
PHYSIOLOGICAL · LABORATORY
UNIVERSITY · OF · LONDON

BIOCHEMISTRY OF MUSCLE AND NERVE

LECTURES ON PHYSIOLOGY, PUBLISHED UNDER THE AUTHORITY OF THE UNIVERSITY OF LONDON

SECOND SERIES

BIOCHEMISTRY OF MUSCLE & NERVE

TEN LECTURES DELIVERED BY
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LONDON
JOHN MURRAY, ALBEMARLE STREET
1904

PREFACE

In the early part of 1903 I delivered a course of eight lectures on the chemical aspect of muscle and nerve physiology at the Physiological Laboratory of the University of London. A little later I received an invitation from the University and Bellevue Hospital College, New York, to deliver there a course of lectures on some subject of chemico-physiological interest. Dr C. A. Herter had for some years been in the habit of giving a course of lectures on Chemical Pathology at this medical school, and when he received the appointment of Professor of Pharmacology at the Columbia University, New York, he desired to perpetuate the course at his old college. This he did by a generous gift, which enables the Bellevue authorities to annually invite some one who has particularly worked at chemical physiology or pathology to deliver such a course of lectures. It is hardly necessary for me to say that I felt highly honoured at the request, that I should be the first Herter Lecturer; and as my lectures at the University of London had not been published, I determined to take the same subject, and the lectures, twelve in number, were given in New York during January of the present year. The little book which I now present is the outcome of these two courses of lectures. For convenience I have divided the subject into ten lectures, the actual order and arrangement being necessarily somewhat different from those which were actually delivered. In New York the longer course enabled me to amplify some of the subjects which I had to pass over rapidly in the lectures given in London. The arrangements of the two laboratories being different, I had also to vary somewhat the

experiments which I selected to illustrate the lectures; but it has obviously become necessary, in writing them out, to amalgamate the two courses so as to make them read as one.

At the University of London, Dr Waller, and in New York, Professor Graham Lusk, placed the resources of their laboratories at my disposal, and it is my pleasant duty to thank them for the assistance they so ungrudgingly gave me.

My thanks are also due to Dr Alcock and to Professor T. G. Brodie, who helped me with my experiments in London; and to Professor Mandel, Dr Arthur Mandel, and Dr Wolf, who performed similar kindly offices in New York.

The publication of this book gives me an opportunity of presenting in a systematised way the numerous researches on muscle and nerve which have been carried out in my laboratory during the past few years, and here again I have to acknowledge with gratitude the help and co-operation of colleagues, friends, and pupils who have worked with me.

I append to this preface a list of the published papers which have been the result of this work, and which have formed the basis on which these lectures have been built.

W. D. HALLIBURTON.

KING'S COLLEGE, LONDON,
1904.

PRINCIPAL PAPERS

THE following list gives the names and places of publication of the principal papers referred to in the following lectures, which have been carried out, either by myself, or in conjunction with colleagues, or under my superintendence. The first six were published during the time I was Assistant Professor of Physiology at University College, London; the remainder were published since I have held the Chair of Physiology at King's College, London:—

1. "An arrangement for determining the Temperature of Heat Coagulation of Proteids," by W. D. Halliburton, *Jour. of Phys.*, vol. iv., 1883.
2. "The Proteids of Serum," by W. D. Halliburton, *Ibid.*, vol. v., 1884.
3. "On Muscle Plasma," by W. D. Halliburton, *Ibid.*, vol. viii.; Preliminary Communication in *Proc. Roy. Soc.*, vol. xlvii., 1887.
4. "Cerebro-spinal Fluid," by W. D. Halliburton, Report of Spina Bifida Committee, *Clin. Soc. Trans.*, vol. xviii., 1885.
5. "Cerebro-spinal Fluid," by W. D. Halliburton, *Jour. of Phys.*, vol. x., 1889.
6. Report on Pathological Effusions, by W. D. Halliburton, *Brit. Med. Jour.*, 1890.
7. "Mucin in Myxœdema, by W. D. Halliburton, *Jour. of Path.*, May 1892
8. "The Chemical Physiology of the Animal Cell," by W. D. Halliburton, Goulstonian Lectures delivered before the Royal College of Physicians, London, *Brit. Med. Jour.*, 11th, 18th, and 25th March 1893.
9. "The Fibres of Retiform Tissue," by R. A. Young, B.Sc., *Jour. of Phys.*, vol. xiii., 1892.
10. "On Fractional Heat Coagulation," by R. T. Hewlett, M.D., *Ibid.*, vol. xiii., 1892.
11. "The Proteids of Nervous Tissue," by W. D. Halliburton, *Ibid.*, vol. xv., 1893.

12. "Note on the Chemistry of Muscle," by Arthur Whitfield, M.D., *Jour. of Phys.*, vol. xvi., 1894.
13. "Chemistry of Connective Tissue," by R. A. Young, M.D., *Ibid.*, vol. xvi., 1894.
14. "Nucleo-albumins and Intravascular Coagulation," by W. D. Halliburton and T. Gregor Brodie, M.D., *Ibid.*, vol. xvii., 1894.
15. "Nucleo-proteids," supplementary paper, by W. D. Halliburton, *Ibid.*, vol. xviii., 1895.
16. "Creatinine in Blood," by P. C. Colls, *Ibid.*, vol. xx., 1896.
17. "Hydrolysis of Glycogen," by M. Christine Tebb, *Ibid.*, vol. xxii., 1897.
18. The Articles entitled "The Chemical Constituents of the Body and Food," and "The Chemistry of the Tissues and Organs," in Schäfer's *Text-Book of Physiology*, by W. D. Halliburton, vol. i., 1898.
19. "An Intestinal Plethysmograph," by A. Edmunds, B Sc., *Jour. of Phys.*, vol. xxii., 1898.
20. "Preliminary accounts of the Physiological Action of Choline and Neurine," by T. W. Mott, M.D., F.R.S., and W. D. Halliburton, *Proc. Phys. Soc.*, February 1897, February 1898, and February 1899.
21. "The Physiological Action of Choline and Neurine," by the same authors. Abstract in *Proc. Roy. Soc.*, vol. lxv., 1899; full paper in *Phil. Trans. of the Roy. Soc.*, series B, vol. cxci., 1899.
22. "Note on the Blood in a case of Beri-beri," by the same authors, *Brit. Med. Jour.*, 28th July 1899.
23. "Observations on the Cerebro-spinal Fluid in the Human Subject," by St Clair Thomson, M.D., L. Hill, M.B., and W. D. Halliburton, *Proc. Roy. Soc.*, vol. lxiv., 1899.
24. The Croonian Lectures on the Chemical Side of Nervous Activity, delivered before the Royal College of Physicians, June 1901, by W. D. Halliburton. Abstracts of the four lectures were published in the *Brit. Med. Jour.*, 15th and 22nd June 1901. The full lectures were published as a separate book by Bale, Sons, & Daniellson, London, 1901.

    Dr Mott's Croonian Lectures on the Degeneration of the Neurone, delivered in 1900 (same publishers), also contained much of our joint work.
25. "The Chemistry of Nerve Degeneration," by F. W. Mott, and W. D. Halliburton. Abstract published in the *Proc. Roy. Soc.*, vol. lxviii., 1901; full paper in the *Phil. Trans. of the Roy. Soc.*, series B, vol. cxciv., 1901.
26. "Regeneration of Nerves," by the same authors, Preliminary Communication, *Annual Reports of the British Association*, Belfast, 1902.
27. "Regeneration of Nerves," by F. W. Mott, Arthur Edmunds, and W. D. Halliburton, Second Preliminary Communication, *Proc. Phys. Soc.*, March 1904.

28. "The Proteids which may occur in Urine," by W. D. Halliburton, *Trans. Path. Soc.*, London, vol. li., 1900.
29. "The Physiological Effects of Extracts of Nervous Tissues," by W. D. Halliburton, *Jour. of Phys.*, vol xxvi., 1901.
30. "The Veratrine-like action of Glycerin," by H. Willoughby Lyle, M.D., *Proc. Phys. Soc.*, January 1901.
31. "The Action of Ether and Chloroform in the Neurons of Rabbits and Dogs," by Hamilton Wright, M.D., *Jour. of Phys.*, vol. xxvi., 1901.
32. "The Action of Ether and Chloroform on the Cerebral and Spinal Neurons of Dogs," supplementary paper, by Hamilton Wright, *Ibid.*, vol. xxvi., 1901.
33. "Specific Gravity of the Brain," by R. H. C. Gompertz, B.Sc., *Ibid.*, xxvii., 1902.
34. "Reticulin and Collagen," by M. Christine Tebb, *Ibid.*, vol. xxvii., 1902.
35. "Fatigue in Non-medullated Nerves," by T. Gregor Brodie, and W. D. Halliburton, *Ibid.*, vol. xxviii., 1902.
36. "The Coagulation Temperature of Cell-globulin and its Relation to Hyperpyrexia," by F. W. Mott and W. D. Halliburton, Mott's *Archives of Neurology*, vol. ii., 1903.
37. "The Choline Test for Active Degeneration of the Nervous System," by F. W. Mott, *Ibid.*, vol. ii., 1903.
38. "Heat Contraction in Nerve," Preliminary Communication ; by T. G. Brodie and W. D. Halliburton, *Proc. Phys. Soc.*, July 1903.
39. "The Precipitation of Proteids by Alcohol and other Reagents," by M. Christine Tebb, *Jour. of Phys.*, vol. xxx., 1904.
40. "Heat Contraction in Nerve," by T. G. Brodie and W. D. Halliburton, in course of publication in the *Jour. of Phys.*

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BIOCHEMISTRY OF MUSCLE AND NERVE

LECTURE I

INTRODUCTORY. COMPOSITION OF MUSCLE

THE rapid growth of the science of Physiology has been accompanied by specialisation on the part of its disciples. This has become necessary because it is impossible for any one person to follow into its many ramifications all the different branches into which Physiology is now subdivided.

One outward and visible sign of this inward manner of growth is the institution of lectures such as those I have the honour of addressing to you now. This comparatively modern departure is, in my opinion, of benefit both to students and to teachers. It enables the taught to hear, from an investigator at first hand, the results of his researches, and to see the main experiments upon which his results depend. It gives a new inspiration to the lecturer to speak to a new class, and to find himself in the surroundings of laboratories other than his own.

Among the special subjects of which various physiologists have made a life-study, that of muscle and nerve is one that has always found numerous adherents. The two tissues are so important, and so closely associated, that this is not surprising. In some hands, the mechanical, in others, the electrical side of this attractive subject has been taken up. The chemical side, however, is the one which has always most interested me, and I hope soon to interest you in it also. A paper on the chemistry

of muscle was the result of one of my earliest researches some years ago; recently, the chemistry of nerve has occupied me and the workers in my laboratory. I want to try and weave these various investigations together, and present you with a connected account of the whole.

The specialisation to which I just referred in speaking of the growth of Physiology is an evil. It tends to narrow the outlook of the investigator. The ultra-specialist is apt to confine himself so closely to his own groove that he forgets to notice what is occurring in the parallel and inter-crossing grooves of others. But those who devote themselves to the chemical side of Physiology run but little danger of this kind. The subject cannot be studied apart from other branches of Physiology, so closely are both roots and branches intertwined. I have entitled these lectures the Biochemistry of Muscle and Nerve, because the work was started from the chemical point of view. But as it progressed, it grew into some channels which are histological, others pharmacological, others still of a purely experimental kind; and in all cases the strictly physiological runs so imperceptibly into the pathological, that any hard-and-fast line between the two is impossible to draw. It will be my duty, therefore, to direct your attention, later on, to subjects which are not chemical at all, or only incidentally so.

Physiology is still in some of the Scotch universities called by its old name, the "Institutes of Medicine." This title embodies an important truth. A medical student should not study Physiology merely to pass a certain examination, and then forget all about it. It is the substratum on which he must subsequently build his knowledge of Pathology and Medicine. I shall endeavour to bear this in mind myself, and point out, as I go along, the pathological and practical bearing of the subjects I bring before you.

I must, however, spend no more time on general reflections such as these. Our time will be fully occupied in the more serious work before us, and I will therefore at once plunge into the middle of things, and ask you to consider with me, for the remainder of the hour, the general composition of the first of the two tissues we are to study, namely, Muscle.

General Composition of Muscular Tissue

Muscle is made up of a number of thread-like structures called muscular fibres, and is subdivided into varieties according to the structure of these fibres. Some are transversely striated, others not. This histological classification corresponds very closely to the physiological subdivision of the muscles into voluntary and involuntary respectively ; the most important exception being cardiac muscle, which, though transversely striated, is nevertheless involuntary.

By far the greatest amount of work in muscular chemistry has been performed upon the voluntary muscles, and it is the composition of this variety that I will ask you first to consider.

Muscle, as usually obtained, is mixed with a certain amount of investing connective tissue, and the gelatin and most of the fat which are mentioned in tables of analyses are derived from this.

I do not want unduly to burden you with figures, plenty of which you will find in your text-books. It will be sufficient at this stage to remind you that muscular tissue contains in round numbers 25 per cent. of solids, and the remaining 75 per cent. is water.

The organic substances contained in the solids are fairly numerous, but the most important and abundant are those of proteid nature. Roughly, 20 out of the 25 parts of solid substance are proteid. Although it is not my intention to deal with questions of dietetics, I may mention in passing that flesh is the most commonly employed source of our nitrogenous food, because it contains proteid both in abundance and in a readily digestible form.

The remaining 5 per cent. of the solids consist of numerous organic materials conveniently grouped together as extractives, and a certain amount of inorganic salts.

The Proteids of Muscle

I shall take first the proteids of muscle, on account of their preponderance both in amount and in importance. Each muscular fibre consists of two parts, a nucleated sheath or

sarcolemma and the contractile substance which it encloses. The sarcolemma is made of a material which resembles elastin in its solubilities, and the nucleo-proteid, which we shall deal with later, is doubtless derived from the nuclei. The main amount of proteid, however, is contained in the semi-fluid, contractile substance. By means of a press, a juice can be squeezed out of perfectly fresh muscles, and this was termed by Kühne the *muscle plasma.* Like blood plasma, this coagulates, and the proteid clot is called *myosin;* when this occurs within the body after death, the stiffening of the muscles known as *rigor mortis* is the result.

Living muscle is alkaline in reaction, but after extreme activity and also after death, the reaction becomes acid; this is in part due to the development of sarcolactic acid.

Our knowledge of the proteids of muscle dates from the investigations of Kühne, who was the first to study muscle plasma with profitable results. He used frog's muscle, which, after having been freed from blood and then frozen, was subjected to strong pressure. The expressed juice was found to be of syrupy consistency, and alkaline in reaction. After lapse of time, especially if the plasma is raised to the temperature of the air, it clots, and the myosin so formed contracts to a slight extent, squeezing out a liquid residue called *muscle serum.* Kühne found this latter fluid to contain a proteid coagulating at 45° C., an alkali-albumin, and an albumin, with salts and extractives in addition. These investigations date a great many years back (1864)—that is, to a time when our knowledge of the proteids was much less than it is at present; there is no doubt that the natural alkali-albumins described by older workers are really globulins.

A good many years later, I was successful in repeating these experiments with mammalian muscle, and was able to show that, not only does cold prevent the coagulation of muscle plasma, but, as in the case of blood plasma, admixture with solutions of neutral salts has the same effect. Addition of water to the salted muscle plasma brings about coagulation (an acid reaction making its appearance simultaneously), and this occurs more rapidly if a solution of "myosin ferment" is

added. I prepared the myosin ferment from muscle in the same way that Schmidt prepared fibrin ferment from blood serum.

Similar saline extracts of muscle which had undergone *rigor mortis* resemble salted muscle plasma very closely; after dilution they undergo coagulation; this at one time I regarded as a recoagulation of the redissolved myosin, and the process is accompanied as before with increase of acidity. Some observers did not consider this to be a true coagulation, but merely a simple precipitation of myosin by dilution with water. This may be true in part, but I am now inclined to look upon the phenomenon not as a recoagulation of myosin which had already clotted, but as a true coagulation (or myosin formation) from the still uncoagulated residue of myosin-precursors. There can, I think, be little doubt that the muscles taken were not completely, but only partially in the state of *rigor mortis*.

The analogy of muscle-clotting to blood-clotting has since Kühne's day been the idea underlying most of the investigations on this subject, and therefore the nomenclature has been similar. Just as the precursor of fibrin in the blood is called *fibrinogen*, so the precursor of myosin in the muscles has been termed *myosinogen*.

We have already seen that in both cases cold and neutral salts will delay clotting. In blood-clotting it is well known that calcium salts are essential, and there are some facts,* though they are not yet fully established, which indicate that this element is also important in the process of muscle-coagulation. The probability also that in both cases one has to deal with the action of a ferment has also been mentioned.

In a recent paper, however, O. Folin † questions the coagulation theory of *rigor mortis* by the following observation. He subjects frogs' muscles to a temperature of -15° C., and this renders them stiff, and irresponsive to stimuli. From these muscles he finds he can prepare muscle plasma in the usual way, which has all the characters of ordinary muscle plasma. That

* Howell and Eaton, *Jour. of Phys.*, vol. xiv., p. 219; S. Locke, *ibid.*, vol. xv., p. 119; W. H. Howell, *ibid.*, vol. xvi., 476; Cavazanni, *Archives italiennes de Biologie*, vol. xviii., p. 156.

† *American Jour. of Phys.*, vol. ix., p. 374.

is to say, muscles which he considers have undergone true *rigor mortis* nevertheless yield muscle plasma which subsequently coagulates. The conclusion I should draw from this work, is not that the coagulation theory is disproved, but simply that cold rigor is not true *rigor mortis* at all. This view is, as a matter of fact, supported by some of Folin's own observations; for instance, there is no formation of acid, and the muscles, though stiff, are still perfectly transparent.

Let me now show you one or two simple but fundamental experiments. The rabbit before you has just been killed. I open the abdomen rapidly and insert a cannula into the aorta, which enables me to wash out with a stream of saline solution all the blood from the muscles of the lower limbs. This being completed, I remove the skin, chop off the muscles, and mince them finely with a mincing machine. The minced muscle is now ground up in a mortar with clean sand, and 5 per cent. solution of magnesium sulphate, and the salted muscle plasma so obtained is filtered off. The filtrate is viscid, and so comes through rather slowly, but quite a small quantity is all we want for the next observation. I will take the few c.c. that are now ready, and test the reaction with litmus, and you see it is still alkaline. I now dilute it with three or four times the quantity of water, and to hurry up the coagulation, place it in the water-bath at 35° C. By the end of the lecture, I expect to be able to show you a typical clot of myosin, which will subsequently contract and squeeze out a salted muscle serum.*

Let me now show you with this other freshly killed rabbit another way of making muscle plasma, which is the method more recently employed by v. Fürth. I obtain the minced, blood-free muscle as before, and grind it up this time with a little physiological salt solution, and some clean sand. I now wrap successive portions in pieces of muslin, and place them in a press. The iron lemon-squeezer I hold is very effective for this purpose. You see the drops of muscle plasma (diluted, of course, to a slight extent with the salt solution added) as they

* This expectation was not fulfilled; the coagulation did not take place until after the lecture was concluded. The specimen was, however, kept and exhibited to the class at the next lecture. The fluid was then distinctly acid to litmus, and by means of Uffelmann's colour reaction, the acid present was shown to be sarcolactic acid.

come through. The muscle plasma is alkaline, viscid, and rather opalescent as before, and filters slowly. Let me, however, with the amount we have now obtained, demonstrate rapidly the process of fractional heat coagulation, a very useful method as a preliminary indication of the existence of more than one proteid in a solution. I place a thermometer in the test-tube, and the test-tube in this flask which is filled with water at about 30° C., and the temperature of which is slowly rising owing to the small Bunsen flame beneath it. You notice no change, but we must watch it carefully as the temperature rises. The temperature is now 42° C., and the opalescence is distinctly deeper; it is now 47°, and the deepening opalescence has culminated in the deposition of flocculi of coagulated proteid; we must keep the temperature constant at 47° C. for a short time, in order to ensure that all the proteid which coagulates at this temperature has separated out. Now we filter it off, and you notice that the filtrate is perfectly clear. We place the filtrate in the test-tube, and place the test-tube in the water-bath again. When we reach 47° no further coagulation occurs, so we continue the heating; now a second crop of flocculi even more abundant than before separates out, and the thermometer reads 56° C. Those of you nearest the table will have noticed that the coagulation with definite flocculi was preceded as before with a gradually deepening opalescence a degree or two lower than 56°.

Another fundamental method for separating proteids from one another, is that which is known as salting out. You will be familiar with the fact that globulins are more readily salted out than albumins. Thus half saturation with ammonium sulphate (one of the most frequently employed of these neutral salts for the fractional precipitation of proteids) will precipitate globulins; complete saturation with this salt is necessary to precipitate albumins. There is no doubt that of the two proteids we have detected by fractional heat coagulation, the one which coagulates at the lower temperature is salted out more readily; both, however, are entirely precipitated by half saturation with ammonium sulphate, and so may provisionally be classed with the globulins. The small amount of albumin left in

the filtrate probably is derived from adherent blood and lymph.

V. Fürth* states he has been able to completely separate the two proteids by the fractional ammonium sulphate method. I have repeated his experiments, and have not succeeded; with a comparatively small amount of salt, the precipitate consists mainly of the proteid which enters into the condition of a heat coagulum at 47° C., but there is always some of the other one as well. On filtering this off and adding more salt, the bulk of the precipitate is the 56° proteid, but mixed with it is the remainder of the first proteid. In other words, the two proteids overlap in relation to the amount of salt added.

Now these two proteids are the precursors of myosin, and so may be included in the general term myosinogen. The one which coagulates at the lower temperature is less important quantitatively, and so I called it *para-myosinogen*, reserving the name *myosinogen* for its more abundant neighbour. Quantitatively, it has been found that the relationship between the two is about 1 to 4 in mammalian voluntary muscle.

The question now arises, Are these two proteids really globulins? There is no doubt that para-myosinogen is a globulin, for not only is it readily precipitable by neutral salts, but it is also insoluble in water, and is, therefore, precipitated by dialysing away the salts that enable it to pass into solution. It is analogous to the cell-globulin, which is found in saline extracts of all protoplasmic structures. Myosinogen, on the other hand, is not a typical globulin, for it is not precipitated by dialysis, that is to say, it is soluble in water.

Are there any other proteids besides these two in muscle plasma or saline extracts of muscle? In my first paper in 1887, I stated there were three others, but all of these are present in minute quantities. These are a globulin, which is coagulated by heat at a temperature higher than the two principal proteids, and an albumin. These, however, are probably due to contamination, with small amounts of blood and lymph. The more thoroughly the blood and lymph are washed away, the

* A good general account of v. Fürth's work is given in his article in the *Ergebuisse der Physiologie*, vol. i., part 1, 1902, p. 110.

less abundant they are. The third of these extra proteids I named myo-albumose, but I soon discovered that there is no albumose in muscle, and Dr Whitfield, in my laboratory, conclusively proved this to be so with the new methods for detecting albumoses, which were available at the time he worked.

In the red voluntary muscles there is a small quantity of hæmoglobin, which we shall deal with when speaking of the pigments of muscle; and in all muscles there is a small amount of nucleo-proteid.

In the involuntary muscles, the phenomena of rigor are much the same as in voluntary muscle. Swale Vincent,* who is the principal worker on this side of the subject, has also shown that the two main proteids are identical. The most striking difference between the two classes of muscle lies in the amount of nucleo-proteid present. It is more abundant in plain muscle than in cardiac muscle, and least abundant of all in voluntary muscle. In other words, those varieties of muscle which in process of development have departed most from the structure of the simple animal cells from which all muscular fibres ultimately develop, are those which possess least of the proteid, which is most typical of simple protoplasmic cells.

This may be very strikingly shown by the experiment I am next going to perform. I have made an extract with a 0.15 per cent. solution of sodium carbonate of equal quantities of the three varieties of muscle. I add dilute acetic acid to the extract of plain muscle, and obtain an abundant precipitate of nucleo-proteid; I do the same with the extract of cardiac muscle, and the precipitate is much less abundant, while with the extract of voluntary muscle, the precipitate is so scanty that those at the back of the room must be content with my word that there is a cloudiness produced.

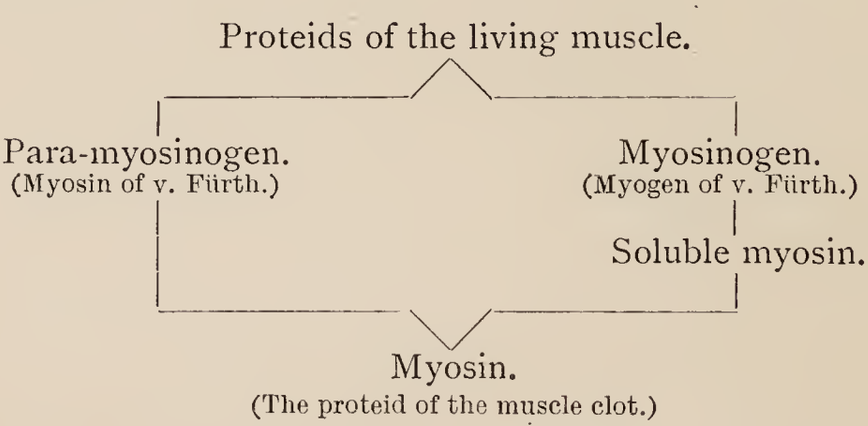
But now we must return to our two principal proteids, and discuss the way in which they pass with the coagulated condition. Stewart and Sollmann† hold the view that the distinction between the two proteids is mainly artificial, and that there is

* *Zeit. f. physiol. Chem.*, 1902, vol. xxxiv., p. 417. Velichi has made similar observations.

† *Jour. of Phys.*, 1899, vol. xxiv., p. 427.

really only one percursor of the clot of myosin. But although the two may, in a sense, be integral parts of a complex proteid, I believe with v. Fürth that the distinction between them is by no means artificial, for the differences between them are many and striking; and Brodie's work on heat rigor, to which I shall have to direct your attention at my next lecture, conclusively proves that they exist as separate entities in the intact muscle.

Whether the transformation is due to ferment action or not, must also be considered in the light of v. Fürth's recent unsuccessful search for the enzyme as unproven. V. Fürth and I, however, are in substantial agreement; the main difference between us is a difference of terms. V. Fürth's names for the proteids have the merit of brevity, but the introduction of new words for things which have previously been known by other names is always confusing to the student. Para-myosinogen passes directly into the clotted condition of myosin or muscle-fibrin; but myosinogen first passes into a soluble condition (coagulable by heat at the remarkably low temperature of 40° C.) before it clots; this soluble stage, which I had noted in my own work, though I failed to give a correct interpretation to it, may be called soluble myosin (v. Fürth's soluble myogen-fibrin). We may put this in a diagrammatic way as follows:—



V. Fürth, in addition to his work on mammalian muscle, has made some interesting comparative observations on the voluntary muscles of cold-blooded animals.

He found that in these (working chiefly with frog's muscle) that the soluble myogen-fibrin just alluded to as a stage in the process of *rigor mortis* in mammalian muscle, is present as

such in the living fresh muscle to a certain extent. He discovered also that in the muscle plasma of fishes there is another peculiar proteid, which he called *myoproteid*. It is precipitable by dialysis and by acetic acid, but is not coagulable by heat.

This work has been extended by Hans Przibram,* who has attempted to classify the animal kingdom on the basis of the muscle proteids. As his conclusions are based on the examination of only thirty species of animals, they may require revision in the future, but such as they are, they are as follow:—

INVERTEBRATES.—Para-myosinogen present; myosinogen absent.

VERTEBRATES.—Para-myosinogen and myosinogen both present.

Fishes: In addition to these two principal proteids, myoproteid and soluble myogen-fibrin occur.

Amphibia: Like fishes, except that myoproteid is present only in traces.

Reptiles, *Birds*, *Mammals*, } Myoproteid absent, and soluble myogen-fibrin only present when *rigor mortis* commences.

It is obviously important that such being the condition of things in the normal state, we should be able to say what occurs in the various pathological conditions of which muscle may be the subject. But here, as is only to be expected in a branch of science so much in its infancy, very little has as yet been done. Indeed, the only research with which I am acquainted is one by Steyrer.† He states that on prolonged tetanisation of rabbit's muscle the amount of para-myosinogen diminishes, that when degeneration occurs after the motor nerves are cut the amount of this proteid increases, and that after the tendon of a muscle has been cut there is no change in the proportion of the two proteids. These investigations open the door to what may prove to be a most profitable line of research, and work such as this may result in a more accurate knowledge of the various degenerative processes which are so frequently seen in muscle after death from various causes.

A few more words on *rigor mortis* will conclude all I shall trouble you with to-day. The onset of post-mortem rigidity is

* *Beiträge chem. Phys. u. Path*, 1902, vol. ii., p. 143.

† *Ibid.*, 1903, vol. iv., p. 234.

not the only problem connected with that subject; * for after a varying interval the rigor passes off and the muscles once more relax; what is the cause of the disappearance of rigor? The usual explanation is that this is due to the onset of putrefactive changes, but the stiffening sometimes passes off too quickly to be attributable to this cause, and I still hold to the opinion that the relaxation is due to the action of an enzyme which produces an autodigestion, and thus a softening of the hardened tissue. There is no longer any reason to suppose that the ferment at work is pepsin which had been previously absorbed from the alimentary canal, for Hedin † has shown that the proteolytic ferment which is present in muscle, as in many other animal tissues (spleen, kidney, etc.), is more like trypsin than pepsin in its mode of action. These ferments, however, are not trypsin, for they differ from the pancreatic ferment by acting best in an acid medium. The conditions for the solution of the coagulated myosin are therefore present, as the reaction of the rigored muscles is acid.

* An interesting side-issue on the subject has been taken up by Fletcher (*Jour. of Phys.*, 1902, vol. xxviii., p 474). He has shown that an abundant supply of oxygen delays the onset of both *rigor mortis* and fatigue.

† *Zeit. f. physiol. Chem.*, 1901, vol. xxxii., pp. 341, 531; *Jour. of Phys.*, 1904, vol. xxx., p. 155.

LECTURE II

HEAT RIGOR OF MUSCLE. EUGLOBULINS AND PSEUDO-GLOBULINS

THE question must have arisen in your minds while I was speaking of the muscle proteids in my last lecture, whether what is found in a saline extract of muscle, or in the muscle juice pressed forcibly from the tissue, really corresponds to what is actually present in the intact muscle itself.

Such an attitude of mind would have been most commendable, for it is only by searching for and meeting any objections which can be raised, that we can hope to arrive at the truth in any scientific problem.

The anatomist is able to see and describe much of the structures of an animal organism without destroying its life. The chemist is able to explore the fields of pure chemistry with his test-tubes and retorts, and is able with precision to discover and formulate natural laws. But it is when the physiologist begins to investigate by chemical methods the realm of living nature, that his great difficulties arise. It is impossible for him to say, for instance, in his observations and experiments with protoplasm, whether what he finds is a true picture of the living substance, or whether he is dealing with the ruins of what he has killed by his reagents. He usually decides in favour of the latter view. If he uses strong and violent reagents, he will assuredly produce more destruction than when he employs gentler measures, and the employment of such a reagent as physiological saline solution is therefore the method which secures the most trustworthy results.

I hope, however, in the case of the muscular proteids, to be

able to convince you that these are not artifacts, but really exist in the muscle itself. The observations upon which I rely to prove this, are those which were first performed by Brodie and Richardson* on heat rigor.

When a muscle is gradually heated, at a certain temperature it is killed, and loses its irritability; at the same time it contracts permanently. This phenomenon is known as *heat rigor*, and is due to the coagulation of the proteid material of the muscle. If a tracing is taken of this shortening, it is found that it does not take place all at once, but in a series of steps, and the various steps correspond to the coagulation temperatures of the various proteids, which may be separated by the process of fractional heat coagulation in a saline extract of muscular tissue. The first shortening occurs at the coagulation temperature of paramyosinogen (47° to 50° C.), and if the heating is continued, a second shortening occurs at 56° to 58° C., the coagulation temperature of myosinogen.

This is very well shown in the tracing (Fig. 1) taken from Brodie's paper. The first contraction began at 43°, became most energetic at 47°, and had finished at 50° C. The length of the muscle then remained stationary until the temperature of 58° C. was reached, when once more you see shortening begins. This tracing illustrates the typical effect of heating a muscle from a warm-blooded animal, and those of you who have the curiosity to consult the paper I have referred to will find numerous other similar tracings which bring out various points of detail.

When, however, we examine the tracings from the muscle of a cold-blooded animal like a frog, we find that there are three instead of two steps in the contraction; they occur roughly at 40°, 47°, and 56° C. You will see how exactly this corresponds with what we have learnt from our previous study of the muscle plasma of the frog; the muscle plasma of the cold-blooded animal contains an additional proteid, namely, soluble myosin, and as this is coagulated by heat at 40° C., it accounts for the

* *Phil. Trans. of the Royal Society*, 1899, B. vol. 191, p. 127. Confirmatory work in the same direction has since been published by Vernon and others in connection with other forms of muscular tissue.

first step in the shortening. This is the experiment I have selected to show you, and we are sufficiently fortunate to have Dr Brodie here to manipulate his own apparatus. We have thought it best to select the frog, because an experiment with the muscles of a warm-blooded animal is a matter of some difficulty to bring off successfully. In warm-blooded muscles it is so difficult to get the experiment under way before the

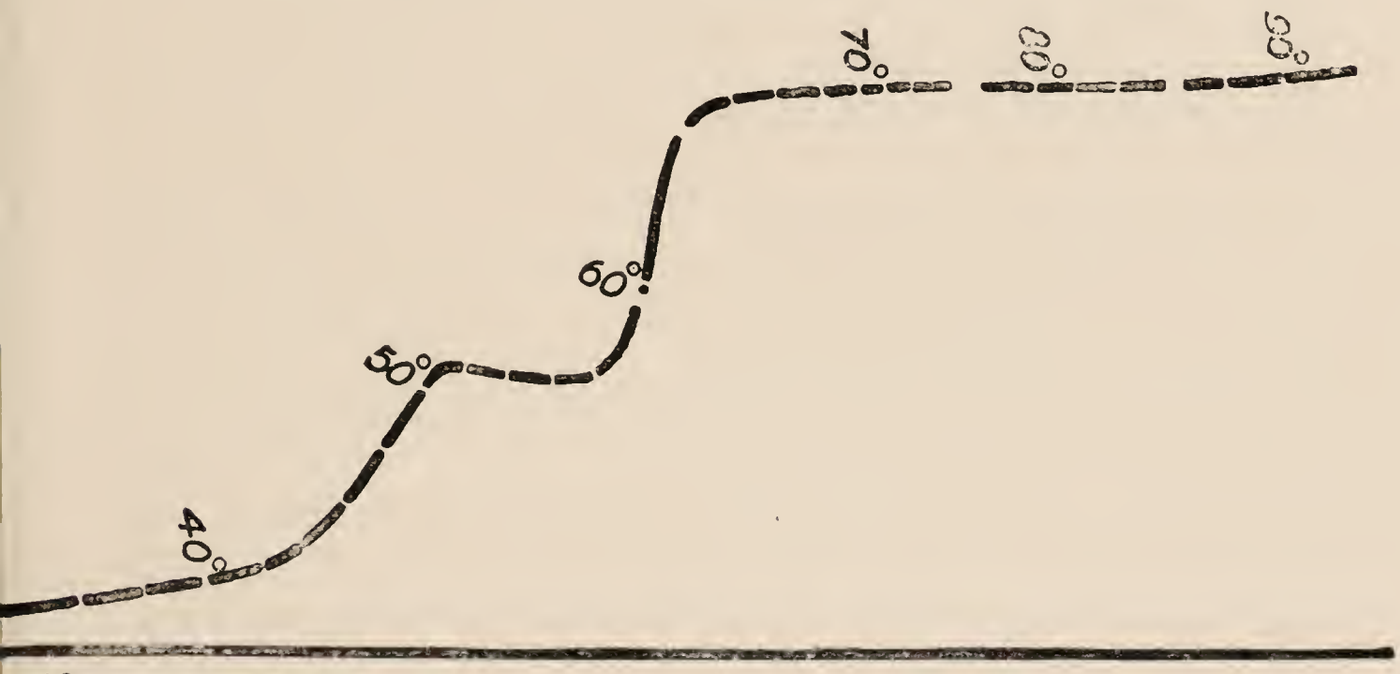


FIG. 1.—Fresh gastrocnemius of mouse. Each break on the curve represents a rise of 2° C. Total duration of experiment, 40 minutes. Magnification, 10. Initial length of muscle, 13 mm. Amount of the first contraction, 1.7; of the second, 2.4 mm. (Brodie and Richardson.)

process of *rigor mortis* sets in, and this naturally upsets any investigation of the myosin-precursors.

It is necessary to select a very slender muscle, in order to ensure that all parts of it are simultaneously at the same temperature, and this obliges us to have apparatus as light, free from friction, and as easily movable as possible.

You see now, the little sartorius in a vessel of salt solution, over a water-bath the temperature of which is going to be gradually raised; the muscle is fixed at its lower end; the upper end is tied to a fine glass thread which pulls down a straw when the muscle shortens. You will be able after the lecture to examine the arrangement by which this straw actuates the

movement of a tiny mirror from which a spot of light is reflected on to this screen. If we wished to obtain a permanent record of the contraction, the spot of light would be made to fall on a slowly travelling photographic plate, and this was the way in which Dr Brodie obtained the tracings of which I have shown you a sample (shown at the lecture as a lantern slide). The breaks on what would otherwise have been a continuous line were produced by means of a little shutter, which at intervals of every two degrees rise of temperature was closed for a few seconds. These breaks render it easy to read the temperature on the tracing.

Dr Brodie's demonstration, however, will to-day be simpler; you will not have to wait until he has developed a photographic plate, but when the lights are turned down, you will see the spot of light travelling up the stationary graduated screen as the muscle shortens. (Dr Brodie then began to heat the water-bath, and called out its temperature, degree by degree.) The temperature of the salt solution by which the muscle is surrounded was originally that of the room 12° C. It has now reached 30° C., and the spot of light is still stationary at the zero mark of the graduation. The temperature is being elevated rather more rapidly than is desirable in an exact experiment, but I have no doubt that this concession to your patience will not prevent you seeing the main phenomena. Anyone who has any experience in determining the coagulation temperatures of proteid solutions, will know that a few degrees before the heat is sufficient to cause the appearance of visible flocculi, there is an opalescent state of the fluid which gradually deepens. You, in fact, saw this for yourselves in my experiment on fractional heat coagulation at my first lecture. I expect we shall see much the same sort of thing in this experiment; although the temperature of coagulation of the first proteid (soluble myosin) is about 40° C., there will be some contraction corresponding to the stage of opalescence before we reach that temperature. My expectation is now being verified; the temperature is now 35° C., and already the spot of light has started slowly on its upward journey, but it is nearly 40° C. before the contraction becomes energetic. The temperature is still rising, but the spot of light is again stationary. Now once

more it begins to move, and the temperature is 45° C. This second contraction is over by the time we reach 50°, and the third and final step in the shortening takes place between 53° and 58° C. You may be a little disappointed at the small amount of shortening that has occurred at the second and third as compared with that in the first step. This, however, is merely due to my desire to make the experiment less tedious. The rapidity of the heating caused such an energetic contraction when the first proteid coagulated, that the effect of the second and third coagulation is rendered comparatively insignificant to look at. If we had allowed time for relaxation to occur after the first contraction, or if we had passively stretched the muscle, the subsequent contractions would have had an opportunity of exhibiting themselves in a more prominent way. We did not like to risk the device of stretching the muscle, because one is so apt to break such a slender object. It gets very brittle after the process of heat rigor has set in. Nevertheless, in spite of these drawbacks, you have seen the main facts verified.

Fig. 2 shows one of Brodie and Richardson's photographic records from a frog's sartorius.

We have hitherto drawn a distinction in the process of heat coagulation between the formation of flocculi and the preliminary stage of opalescence. There is no real distinction between the two. One merges into the other imperceptibly. If one finds in a solution of a proteid that opalescence begins at a certain temperature, and the separation of flocculi at a higher temperature, this does not mean that the phenomenon at the higher temperature is of a different nature from that which occurs at the lower; for if the heating is continued long enough, flocculi will form at the lower temperature. Hewlett* puts the explanation of this very well as follows:—

"In an aqueous solution of a proteid even when concentrated, there are but comparatively few molecules of proteid. The temperature of the solution represents the *average* energy of all the molecules. When opalescence appears, we may suppose that the changes which convert soluble into coagulated proteid have taken place in some proteid molecules, there being a few

* *Jour. of Phys.*, 1892, vol. xiii., p. 496.

which happen to have attained greater velocity than that represented by the average. Owing to the irregular collisions of the molecules, their energies or velocities are constantly changing, and in time each proteid molecule will in its turn attain the

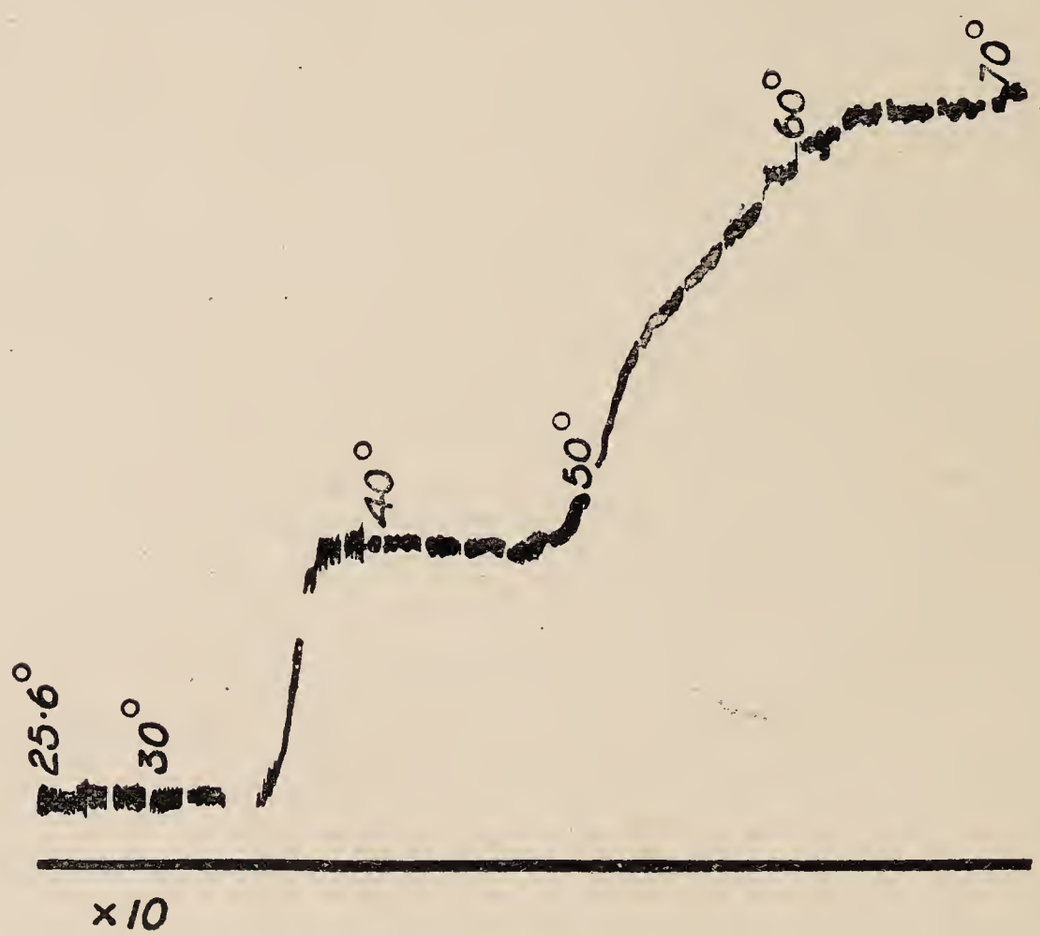


FIG. 2.—Frog's sartorius which had been heated to 34° C. for sixty-five minutes twenty-four hours previously. It was then kept in cold dilute blood, and on the following morning, although somewhat opaque, it was found to be still irritable. It was then passively stretched, and the above tracing taken. Each break in the curve records a rise of 2° C. There is a contraction commencing at 34° C., although much less than that given by a fresh muscle. A second contraction begins at 47°-48° C., which is more marked than in a fresh muscle, because of the preliminary stretching. On account of the large amount of contraction previously produced, the third and final shortening at 56°-58° C. is not well-marked. (Brodie and Richardson.)

velocity necessary for coagulation to take place. The more dilute the solution the longer will this take, and in any case the process must be a slow one, on account of the comparatively small number of proteid molecules. Raise the temperature a degree or two, and the number of molecules at any given time which have attained the velocity necessary for coagulation to

take place is much increased, and coagulation becomes more rapid. Now flocculi are merely aggregations of fine particles, these being aggregations of molecules, and if the particles form at a rapid rate, then flocculi soon appear, as is the case when the temperature is raised above that of opalescence, while if the temperature is maintained at that of opalescence, particles form only slowly, and it will be a long time before flocculi are seen."

We have noticed that in experiments like those of Brodie on heat rigor there is corresponding evidence that coagulation is a gradual, not a sudden, process, and when we remember that the contractile substance of muscle is a semi-fluid material, we can at once apply Hewlett's explanation to it also.

The temperature at which the separation of flocculi occurs when a solution of soluble myosin is heated at the usual somewhat rapid rate is 40° C., but Brodie and Richardson found that in saline extracts of frog's muscle, the soluble myosin can be completely coagulated at 34.5° C., provided the temperature is maintained at that point for a sufficient length of time. In a corresponding way they showed that in an experiment on heat rigor the first step in the shortening can be entirely finished at a temperature of 35°, provided again sufficient time is given.

This consideration is not merely of theoretical interest; it is one of immense practical importance, and I will ask you to bear it in mind until we come later on in our study of hyperpyrexia to one of its applications.

There is another point of still greater interest arising out of Brodie's work on heat rigor, which is this. What temperature is necessary to destroy the irritability of a muscle? Must we cook it so thoroughly that all its proteids are coagulated? or is it only necessary to raise the temperature sufficiently high to coagulate the proteid which has the lowest temperature of heat coagulation? Or is the requisite temperature somewhere between the two extremes? The answer to this question is that the muscles lose their irritability after the first step in the shortening has occurred. We see, therefore, that when one of the muscular proteids has been coagulated, the living substance as such is destroyed. It is therefore incontrovertible that

although it is convenient, and from some points of view instructive and necessary, to regard the muscle proteids as separate units, they are not really independent. The unit is protoplasm, and if one of its essential constituents is destroyed, protoplasm as such ceases to exist.

You will realise without waiting for me to discuss the harmful influences of extremely high body temperatures, that this is a point for the practical physician; it is also of interest to the comparative physiologist, and is an evidence how different kinds of organisms are fitted to their environment. Frogs are much more easily killed by an elevated temperature than warm-blooded animals, simply because their living tissues contain a proteid which coagulates so easily under the influence of heat. If a man were suddenly provided with frog's muscles, and his normal temperature of 37° C. was still maintained, you can understand that he would not live long. On the other hand, a bird has a normal temperature much higher than that of a man, namely about 42° C., which is getting dangerously near to the coagulation temperature of para-myosinogen, but in the bird heat rigor does not occur until the temperature is raised to 53° C. It is evident that in the comparatively elevated temperature necessary to cause the first coagulation in birds' muscle plasma we have another instance of biological adaptation.

Since the foregoing was written, Simin * has performed similar experiments on the heat rigor of heart muscle. In frog's heart muscle, the first step in the process occurs at 43° C., whereas in mammals it occurs at 46° C. After *rigor mortis* has set in, the first step in both cases is abolished. Brodie also found the absence of the first contraction the most marked feature which distinguishes rigored voluntary muscle from living muscle.

Euglobulins and Pseudo-Globulins

For the remainder of the hour, I will ask you to consider with me another question which is related to a study of the muscle proteids. The close connection between physiology and pathology I have already alluded to, and, as a rule, a knowledge of pathological processes is a sequel to that of the normal. But occasionally it is the other way round, and from what I

* *Centralb. f. Phys.*, 1904, vol. xviii., p. 89.

have now to say, you will understand how the consideration of a pathological problem led me to take up a physiological question.

A few years ago I was called upon to open a discussion at the Pathological Society, London, on the proteids which may occur in the urine, and I took occasion to point out that the presence of albumin in the urine in greater quantity than the globulins of the blood when the damage to the kidney cells is comparatively slight may possibly be a question of molecular size. Experiments by Gottwalt* had previously shown that albumin passes under pressure through the membrane of a dialyser more readily than globulin, and Dr T. G. Brodie stated at the discussion to which I have just referred, that some experiments he had performed on perfusion of the isolated kidney supported the theory that the molecules of the globulins are larger than those of the albumins.

Considering the difficulty of making direct observations on the molecular size of proteids, it is important to utilise all indirect methods for this purpose. The globulins are, as is well known, more readily salted out of their solutions than the albumins; they coagulate, as a rule, at lower temperatures, and some of them, like fibrinogen and para-myosinogen, pass easily into insoluble modifications. All these facts point to an extreme colloidal condition.

If now we compare the colloidal with the crystalline carbohydrates, we see that the colloidal members of the group are readily salted out from solution, whereas the crystalline ones are not. The dextrins which occupy an intermediate place between the two divisions are less readily salted out than the colloidal carbohydrates, like starch and glycogen. The facility with which the carbohydrates are precipitable by alcohol runs parallel to this; starch and glycogen require a comparatively small amount of alcohol to precipitate them; the dextrins require more, while the crystalline carbohydrates or sugars are soluble in alcohol.

It therefore became interesting to ascertain whether the proteids form a series comparable to that described in the

* *Zeit. f. physiol. Chem.*, vol. iv., p. 423.

case of the carbohydrates. The difficulty of precipitating the proteids whose molecules are known to be comparatively small (proteoses and peptones) by means of alcohol is a matter of common experience, and some members of this group derived from the vegetable world are even stated to be soluble in alcohol. If, therefore, it can be shown that the globulins require a lower percentage of alcohol to precipitate them than the albumins, we have an additional piece of evidence in favour of the view that the globulins have larger molecules than the albumins. This is the question which I asked Miss Tebb to investigate in my laboratory, and it will be seen that the answer obtained was an affirmative one.

Since this work was started, a paper appeared by Pauli and Rona,* who also pointed out the correspondence between the precipitation of colloids by alcohol and by salts, but actual quantitative results, such as Miss Tebb obtained, are not given.

The experiments were carried out with four typical groups of proteids, namely, those of blood, of egg-white, of milk, and of muscle. It is the inclusion of the muscular proteids in this research that has led me to give here a general account of the results obtained.

In the case of the blood, the proteids present in solution are: (1) fibrinogen, the globulin which is the precursor of fibrin; (2) serum globulin; and (3) serum albumin. The second and third in this list are present in the serum, after blood-clotting has taken place. Many years ago, I pointed out that the u bstance called serum albumin of some animals can be separated by fractional heat coagulation into two, or in some cases into three proteids; but as it is difficult to find any further difference except that of heat coagulation temperature between them, the question whether they are really distinct proteids or not is still *sub judice;* and for our present purpose, serum albumin may be provisionally regarded as a single substance.

More recent research has shown that serum globulin is not a single proteid. The proteid which is salted out from serum by means of half saturation with ammonium sulphate, or complete saturation with magnesium sulphate really consists of

* *Beiträge chem. Phys. u. Path.*, 1902, vol. ii., p. 1.

two proteids; one of these is insoluble in water, that is to say, it is precipitable by dialysing away the salt from its solutions: it therefore fulfils all the definition of a true globulin, and is called *euglobulin*. The other, known as *pseudo-globulin*, is not precipitable by dialysis.

In the investigation of serum quantitatively, it is now usual to estimate the proteids in three fractions: euglobulin, as one would expect, is most readily salted out, and is brought down by one-third saturation with ammonium sulphate; in the filtrate, pseudo-globulin is precipitated by raising the amount of salt to the point of half saturation; the third fraction consists of serum albumin, full saturation with the salt being necessary to throw it out of solution.

I need hardly assure you that these refinements are not invented by physiologists for the purpose of making the student's life more irksome. As knowledge progresses, it often becomes more complex for a time. This is not the occasion for one to describe recent researches on the blood in relation to the subject of immunity and similar questions. I will only say that the proteid-like substances known as antitoxins, precipitins and the like have been to a certain extent separated out by fractional precipitation with salts, for they tack themselves on to the various proteid fractions in different ways. This line of research, still in its infancy, holds out great promise for the future.

Miss Tebb soon found as her work progressed that the globulin of white of egg must also be divided into an eu- and a pseudo- fraction.

With this introduction we may now pass to her results. The following numbers represent the amount of alcohol per cent. necessary to produce complete precipitation of the various proteids.

Blood Proteids—

Fibrinogen	30
Serum euglobulin	25
Serum pseudo-globulin	50
Serum albumin	50

Egg-White Proteids—

Egg euglobulin	20
Egg pseudo-globulin . . .	65
Egg albumin	40

Muscle Proteids—

Para-myosinogen	20
Myosinogen	80

Milk Proteids—

Caseinogen—The main bulk is brought down by 45 per cent. of alcohol, but it is not completely precipitated by 90 per cent.

Lact-albumin—The main bulk is brought down by 65, and the last traces by 80-85 per cent. of alcohol.

We therefore see from these results :—

1. That the true globulins of blood and egg-white require considerably less alcohol to precipitate them than do the albumins.

2. Although the pseudo-globulins are more readily salted out from their solutions than are the albumins, and less readily than the euglobulins, the precipitability by alcohol does not run quite parallel to this. On the whole the pseudo-globulins resemble the albumins in their precipitability by alcohol, but in one case, that of egg-white, the albumin is more readily precipitable by alcohol than is the pseudo-globulin.

The results obtained with the proteids of milk were a little unexpected: lact-albumin is precipitable with difficulty by alcohol, and so falls into line with the other albumins; but caseinogen, which one would rather have anticipated to behave like a globulin, requires also a considerable amount of alcohol to precipitate it entirely; most, however, is thrown out of solution by a comparatively small amount of alcohol.

The results with the muscle proteids are those which will interest us most. Both (and especially para-myosinogen) are readily salted out from their solutions, but, as we have already seen, myosinogen is soluble in water, and we have described it as an atypical globulin. It is not stretching the use of terms too

much to say that para-myosinogen is the euglobulin of muscle; like the other euglobulins, it is readily precipitable by alcohol; myosinogen, the pseudo-globulin of muscle, requires much more alcohol to precipitate it entirely.

The main conclusion drawn from this work is, that there is distinct though indirect evidence that the true globulins have larger molecules than the pseudo-globulins and albumins.

Among some subsidiary points worked out by Miss Tebb, the following is of importance :—The prolonged action of alcohol renders proteids insoluble. The euglobulins are most readily rendered insoluble in this way; the pseudo-globulins and caseinogen come next, whilst of the proteids investigated the albumins are the most difficult to convert into insoluble modifications by alcohol. It was previously well known that proteids of still smaller molecular size (proteoses and peptone) not only require a large amount of alcohol to precipitate them entirely, but also are not rendered insoluble by prolonged contact with that reagent.

Considering the way in which this investigation originated from a study of proteids in pathological urine, it is interesting in conclusion to note some results published last year by J. Joachim.* He finds that when serum globulin occurs in urine, it is almost exclusively pseudo-globulin which is present.

* Pflüger's *Archiv*, 1903, vol. xciii., p. 558.

LECTURE III

THE PIGMENTS OF MUSCLE. PROPERTIES OF NUCLEO-PROTEIDS. THE FERMENTS OF MUSCLE

THE voluntary muscles of a mammal are usually divisible into two main varieties, which are respectively called the pale and the red muscles. These are readily distinguishable to the naked eye by their colour, to the microscope by certain points of histological difference, and in physiological experimentation it is found that the contraction period is longer in the red than in the pale muscles.

The rabbit is generally used as the animal in which to show this in demonstrations, because in this animal the distinction is so well seen; the majority of the muscles are of the pale variety, but in a few muscles the fibres are almost exclusively of the red variety. I have here the leg of a rabbit from which all the blood has been washed out by a stream of salt solution. We have therefore excluded any coloration due to the blood. If you look at it, you readily see how such muscles as the semi-membranous and crureus stand out red on the pale background of the surrounding flesh.

I have taken a small piece of one of these red muscles, and after mincing it, ground it up with some salt solution; the filtered extract I have placed in front of a spectroscope, and after the lecture you will have the opportunity of identifying the two well-known absorption bands of oxyhæmoglobin.

There is no doubt that the coloration is due to blood-pigment actually contained within the muscle plasma.

There is some colour in the so-called pale muscles, and

probably here we have to deal with the same pigment in smaller quantities.

Some years ago, however, Dr MacMunn* stated that the main pigment of muscles is a special colouring matter, to which he gave the name *myohæmatin*. He found it also in many invertebrate animals, in the blood of which no hæmoglobin is contained. He found also in other tissues somewhat similar pigments, and the name *histo-hæmatins* which he gave to the whole group indicates their similarity to hæmoglobin; he further considered that they act as respiratory pigments, and showed in support of this view that their absorption bands undergo changes on oxygenation and reduction.

Unfortunately, these pigments were never separated out from the tissues in a pure condition and analysed. They were in the main detected and identified by the spectroscope, usually after the tissues had been rendered transparent by the action of such reagents as glycerin.

I will next demonstrate to you the method by which myohæmatin can be rendered visible. I have here a piece of the pectoral muscle of a pigeon, a very deeply pigmented muscle. It has been in glycerin since yesterday, and is now quite translucent; I place a thin layer of it between two glass slides and put it in front of the spectroscope. After the lecture you will be able to see its absorption bands, which are shown in the accompanying diagram (Fig. 3, spectrum 1).

MacMunn proved that the bands fade on the application of a reducing agent.

He introduced also another method by which the pigment may be obtained in solution, which I will proceed to show you. Again I have taken the pigeon's breast muscle, chopped it into small pieces, and placed it in a flask with excess of ether since yesterday. You will see that the ether is of a distinctly yellow colour; but the yellow pigment is not myohæmatin, but is simply a fatty pigment or lipochrome. These lipochromes used at one time to be called luteins, because the best known member of the group was obtained from the corpus luteum of the ovary. Adipose tissue, milk, blood serum, etc., yield similar pigments

* *Phil. Trans.*, 1886. *Jour. of Phys.*, 1887, vol. viii., p. 51.

to solvents of fat. The lipochrome now dissolved in the ether doubtless comes from the adipose tissue and blood serum contained within the muscle.

I pour off the yellow ethereal fluid, and now, when I am reaching the bottom, another fluid on which the ether has been floating comes away. The colour of this aqueous fluid is reddish; it is rather cloudy, for it contains in suspension a number of particles of minced muscle. I proceed now to separate the two fluids with a separating funnel, and we will examine the red aqueous fluid, for it is this that contains myohæmatin. In contact with ether, osmotic processes have occurred in the muscle, resulting in the passing out of it of this watery fluid, which carries some of the pigment with it. It also contains a certain amount of proteid and extractives, but those need not concern us. After filtration, the fluid is seen to be perfectly clear. I place some of it in front of another spectroscope, and those of you who take the trouble of examining it, will see the absorption bands figured in spectrum 2 of Fig. 3. If you are familiar with the spectroscopic appearance of hæmoglobin derivatives, you will observe that the two bands seen are something like those of hæmochromogen, but are nearer the violet end of the spectrum. This substance further differs in spectroscopic characters from the myohæmatin, as seen by the glycerin method, and so was termed "modified myohæmatin" by MacMunn. A similar modification is produced by subjecting the muscle to artificial gastric digestion.

The question before us is, whether myohæmatin is modified hæmoglobin. Levy,* who worked under Hoppe-Seyler's direction, gave it as his opinion that myohæmatin is simply hæmochromogen. It is difficult to understand how he can have reached this conclusion, for, seeing that spectroscopic analysis was the only method employed, it is remarkable that he did not pay attention to the marked difference in the position of the absorption bands in the two cases. The difference is so evident that you will, even with the small direct vision spectroscopes on the table, have no difficulty in seeing it. I have placed some

* *Zeit. f. physiol. Chem.*, vol. xiii. MacMunn's reply is in the same volume.

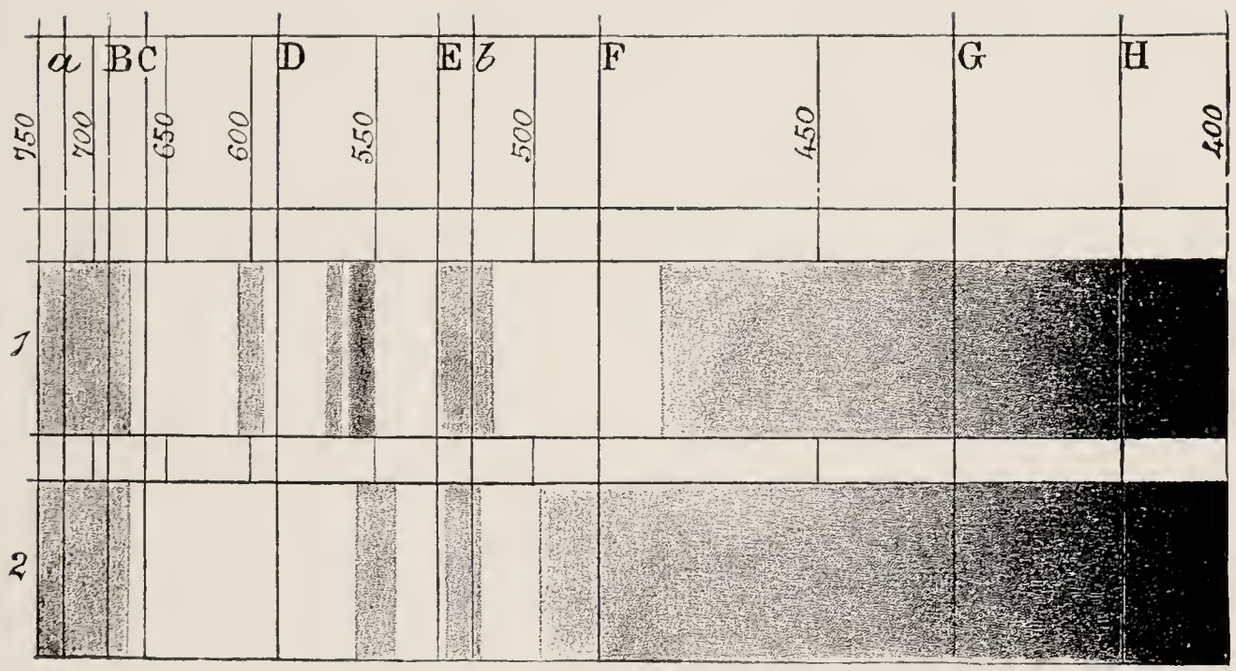


FIG. 3.—1. Absorption spectrum of myohæmatin in muscle rendered transparent by glycerin. 2. Absorption spectrum of modified myohæmatin obtained by ether method.

[*To face page* 28.

hæmochromogen prepared from blood in front of another spectroscope to enable you to compare the two.

Mörner * has suggested that the proteid constituent of the pigment may be different in the two cases, but this is only a hypothesis. The subject is one which would repay further research. I think myself that there can be no doubt that myohæmatin is a derivative of hæmoglobin, but whether the muscular tissue is capable of producing the change in spite of the reagents added, or whether the reagents added are mainly responsible for the change, one cannot at present say. I am led to this conclusion by the following consideration. The muscles in which I have been able to show you the pigment were not freed from blood before being placed in glycerin or ether respectively; and they contained plenty of blood at the start. Yet, the spectrum of the blood pigment has entirely disappeared, and its place taken by that of myohæmatin.

I am strengthened in this conviction by some experiments of Copeman.† He mixed defibrinated and slightly diluted blood with minced muscle, and kept the mixture at 36° C. for nearly three weeks, contact with air being prevented. At the end of that time, the spectrum was not distinguishable from that figured by MacMunn for myohæmatin. On heating to near boiling-point the bands disappear; and this, I had previously pointed out, was a distinctive feature of modified myohæmatin. Similar experiments carried out with small quantities of liver and other tissues macerated in blood resulted in the formation of the hæmoglobin derivative nearest, spectroscopically, to myohæmatin, namely, hæmochromogen.

Further, MacMunn himself has shown that hæmoglobin derivatives like acid hæmatin and hæmatoporphyrin can be obtained from myohæmatin.

Properties of Nucleo-Proteids

The mention of nucleo-proteids among the constituents of muscle leads me next to speak in general terms of these impor-

* *Nord. med. Ark.*, Stockholm, Festband, 1897.

† *Proc. Phys. Soc.*, Nov. 8, 1890. *Jour. of Phys.*, vol. xi., p. xxii.

tant substances. They are not very important in muscle from the quantitative point of view. But as they are also found in nervous structures, I want briefly to indicate some of their principal characters before we finally leave the subject of the muscular proteids.

I then described these substances, dealing first with their chemical characters and relationships, and the purine and other substances obtained ir the decomposition of true nuclein in contradistinction to that of pseudo-nuclein I then proceeded to deal with the physiological importance of nucleo-proteids, and dwelt particularly on the way in which, when injected into the blood stream, they cause intravascular clotting. This led me finally to a demonstration. I prepared some nucleo-proteid from a freshly removed kidney before the class, by my sodium-chloride method, dissolved it in dilute sodium carbonate solution, and injected it into the blood stream of an anæsthetised rabbit. After the death of the animal, which occurred after the injection of a few cubic centimetres, the intravascular coagulation was found to be very complete, especially in the venous system. This necessarily led me to speak on the subject of blood coagulation in general. These subjects, although appropriate enough in a spoken lecture and demonstration, were introduced by way of parenthesis ; but it is neither suitable nor necessary to enter into these side questions in a book which professes to deal especially with the chemistry of muscle and nerve.

The Ferments of Muscle

Ferments are substances which have to a great extent eluded the grasp of the chemist. All he can say is, that they are probably proteid-like in nature, and in some cases the proteid material with which they are either identical or united, is, as in the case of the fibrin ferment, of the nucleo-proteid variety.

I have already alluded to the possible existence of a myosin ferment concerned in muscle coagulation. I will only add that if it does exist, it is not identical with fibrin ferment. Fibrin ferment does not hasten the clotting of muscle plasma, nor does myosin ferment hasten the coagulation of blood plasma. The addition of pieces of fresh muscle and fresh tissues generally does accelerate the clotting of blood, or of blood plasma, and this is, according to some observers, due to adherent lymph ; this view is disputed by Delezenne, and the actual substance to which the result is due must be considered unknown at present.

In addition to this ferment, let me also remind you of the existence of a proteolytic enzyme which, I have already stated, is probably concerned in the disappearance of *rigor mortis* (p. 12).

In addition to these, there are two others: (1) an amylolytic ferment which converts starch into sugar; here again muscle is not peculiar, but a similar enzyme is obtainable in extracts of most tissues. The final sugar formed is dextrose; hence it is also necessary to assume the existence of another ferment (maltase), which converts maltose into dextrose; (2) a glycolytic, or sugar-destroying ferment, which is probably of importance in carbohydrate katabolism, and to which Brunton and Rhodes* were the first to draw attention. Glycolysis occurs in many tissues, and the agent or ferment to which this is due, is believed by Cohnheim† to be rendered active by the internal secretion of the pancreas.

* *Proc. Roy. Soc.*, 1901, vol. lxviii., p. 323.

† *Zeit. f. physiol. Chem.*, 1903, vol. xxxix., p. 336. See also J. Feinschmidt (*Beiträge chem. Phys. Path.*, 1903, vol. iv., p. 511), on the Sugar-destroying Ferment in Organs.

LECTURE IV

THE EXTRACTIVES AND SALTS OF MUSCLE

THE extractives of muscle form a heterogeneous group of organic substances, which occur in small quantities, and which may be extracted by the usual reagents employed for that purpose, such as alcohol, ether, or water.

I will begin by giving you a list of the extractives, putting them in two groups, non-nitrogenous and nitrogenous.

A. Non-nitrogenous extractives.

1. Glycogen.
2. Dextrin and sugars.
3. Lactic acids.
4. Inosite.
5. Fat.

B. Nitrogenous extractives.

1. Creatine.
2. Creatinine.
3. Xanthine.
4. Hypoxanthine.
5. Uric acid.
6. Urea.
7. Carnine.
8. Carnic acid.
9. Inosinic acid.
10. Taurine.

Nearly all of these possess some special point of interest. Many of them, like glycogen, sugar, creatine, and urea open up important general questions of metabolism. Some, like uric acid and its near chemical relatives, would lead us into pathological bypaths if we followed them up in detail. To treat all of these questions in full would lead us too far from the

main study in which we are engaged, and exigencies of time further compel me to do little more than indicate the main characters of each member of our long list, and to refer you to the side questions which they suggest.

The Carbohydrates of Muscle

The principal carbohydrate of muscular tissue is glycogen. It may be extracted from muscle by boiling-water (Brücke), by dilute potash (Külz), or by strong potash (Pavy, Pflüger). The last-named method is the one now most generally employed, and Pflüger has fully confirmed Pavy's statement that the extraction by the use of this reagent is not only very thorough, but there is little or no loss of glycogen during the process. In a number of somewhat lengthy papers in recent volumes of Pflüger's *Archiv*, it has been shown that the estimations previously made by the older methods require revision, and that glycogen is a constituent of many tissues and organs where its presence was previously unsuspected. The addition of a comparatively small amount of alcohol to the extract precipitates the glycogen, and so it may be separated from the proteid matter in the extract, which requires more alcohol to precipitate it.

Glycogen is not equally distributed throughout the musculature, and estimations have been made in various skeletal muscles and involuntary muscles by Cramer, Boruttau, Chittenden, Bizio and others. The numbers obtained possess but little general interest. It is more important to study the varying amount under different circumstances.

1. *In starvation.*—The muscle glycogen disappears during inanition, but much more slowly than the hepatic glycogen.*

2. *During work.*—The glycogen of the muscles disappears during work, being probably transformed into sugar which

* Weiss, *Sitzungsber d. k. Akad. d. Wissensch.*, Wien, vol. 64 ; Aldehoff, *Zeit. f. Biol.*, vol. xxv., p. 137 ; Luchsinger (*Dissertation*, Zurich, 1875) stated the heart glycogen disappears more slowly still, but Aldehoff could not confirm this.

then undergoes combustion. The following table* may be taken as a type of the results obtained.

	Percentage of Glycogen.		Percentage loss of Glycogen in Tetanised Limb.
	In limb at rest.	In the opposite limb, made to contract for 25 to 65 minutes.	
1	0.1277	0.114	12.76
2	0.2287	0.1942	15.09
3	0.2267	0.1917	15.44

This question is one of great importance, and opens up the general question of the source of muscular energy. There are some physiologists who still hold with Liebig that the proteids are the main source of muscular energy, whereas the exactly opposite view is held by others, namely, that the non-nitrogenous constituents are those which are chiefly used up. The large and immediate increase in the discharge of carbon dioxide which occurs when a muscle contracts, is certainly in striking contrast with the unimportant increase in the products of proteid katabolism which takes place, as was first shown by the classical experiment of Fick and Wislicenus in their historical ascent of the Faulhorn in 1865.

Those of you who are athletes should be specially interested in this problem. As a rule, an athlete does not trouble much about the scientific reasons for his method of training; he is guided in his diet by what others have found to be successful in the past, and a good deal of tradition gets mixed with the ideas that prevail on the subject. Generally, a large amount of proteid food is taken, and carbohydrates and fats are knocked down. Experience shows that muscle works most economically when fed chiefly on proteid; at any rate, the waste in heat production that accompanies the combustion of fat and carbohydrate is not so great. We must, however, remember that during recent years, feats of great endurance have been performed by men who have used mainly carbohydrates during training. On most

* Manché, *Zeit. f. Biol.*, vol. xxv., p 163.

subjects of physiology, as in politics, where there are divergencies of opinion, there is generally truth on both sides. I do not intend to weary you with the details of this particular controversy, but will merely say that the majority of level-headed physiologists are now agreed that muscular energy comes from all three classes of food stuffs. So long as an ordinary mixed diet is taken, the diet which centuries of experience have shown to be most suitable for man, there will be sufficient carbonaceous food present in the muscle, and the muscle chiefly uses this for the energy required in its contraction. But if this is not given, or the accumulated store of fat and carbohydrate in the muscle is used up, then muscular work must be maintained by the disintegration of its proteid.*

3. *Paralysis* of a muscle, produced either by cutting its nerve † or its tendon,‡ causes an accumulation of glycogen in it. This is the opposite to the effect of work, and so would have been expected. Ligature of the arteries to a muscle leads, on the other hand, to a decrease in the glycogen, especially if œdema follows the operation, the accumulated lymph leading to saccharification (Manché ; Chandelon).

4. *Removal of the liver.*—Though some observers § consider that the muscles have a glycogenic function independent of that of the liver, it appears to be an undoubted fact that extirpation of the liver leads to a rapid disappearance of the muscular glycogen (Minkowski).‖

The sugar in muscle is during life at a minimum ; as it is formed from the glycogen, it is apparently soon burnt up. After death, as in the liver, the amount of sugar increases as the

* On the beneficial effect of feeding voluntary muscle on sugar, see Lee (*Centralbl. f. Phys.*, 1901, vol. xv., p. 482). Corresponding effects on the isolated mammalian heart are described by Locke (*Proc. Phys. Soc.*, 1904, p. xii. ; *Jour. of Phys.*, vol. xxxi.). The important sugar is dextrose.

† Chandelon, Pflüger's *Archiv*, vol. xiii., p. 626. See also Manché, *loc. cit.*

‡ E. Krauss, Virchow's *Archiv*, vol. cxiii., p. 315.

§ Prausnitz, *Zeit. f. Biol.*, vol. xxvi., p. 377. Schmelz, *ibid.*, vol. xxv., p. 180.

‖ *Arch. f. exper. Path. Pharm.*, vol. xxiii., p. 139. See also Schmelz, *loc. cit.;* and Laves, *Inaug. Dissertation*, Konigsberg, 1886.

glycogen disappears. Nasse* considered that the sugar is maltose; but the work of Panormoff† with the phenylhydrazine reaction showed it to be dextrose. That dextrose should be found is not to be wondered at, for in my enumeration of the ferments in muscle you will remember I drew your attention to an amylolytic enzyme, and a maltase as well. Some few years ago, Miss Tebb, in my laboratory, worked out the properties of the dextrins which are formed as intermediate products in the conversion of glycogen into sugar, and showed that on the whole they are analogous to those found during the hydrolysis of starch. We should therefore expect in muscle to find not only the final product dextrose, but also the intermediate stages of dextrin and maltose. The careful work of Osborne and Zobel‡ has in my opinion placed it beyond doubt that this is really the case. Although it is therefore correct to use the word sugars in the plural in reference to muscle, we ought not to include inosite among them. Inosite was formerly called muscle-sugar, and is present. But, as you all know from your text-books, inosite, though isomeric with the glucoses, is really a member of the aromatic series.

The Fat of Muscle

Fat is always obtainable from muscular tissue, though whether it occurs in the true muscular substance apart from the entangled adipose tissue, it is difficult to say. Dormeyer§ finds that after muscle has been subjected to preliminary gastric digestion, ether extracts 8.5 per cent. more of the total fat obtainable, and has gone so far as to say that without such preliminary digestion, extraction is useless for quantitative purposes. This view has not been entirely confirmed by subsequent workers; if the subdivision of the muscle is great enough, and the extraction carried out sufficiently long, with suitable agitation during the process, ether has been found

* *Zur. Anat. u. Phys. der quergestreiften Muskel*, Leipzig, 1882.
† *Zeit. f. physiol. Chem.*, vol. xvii.
‡ *Jour. of Phys.*, 1903, vol. xxix., p. 1.
§ Pflüger's *Archiv*, 1896, vol. lxv., p. 90.

sufficient. There is, however, no doubt that gastric digestion is one means of accomplishing sufficient subdivision. Bogdanow * believes that the fat thus soluble in ether with difficulty is a real constituent of the muscle plasma, and states that it is richer in volatile fatty acids than that from the surrounding connective tissue.

The only other point of importance in reference to this question is the statement by Leathes,† that the red muscles of the rabbit are richer in fat than the pale muscles of the same animal, or the mixed muscles of the cat.

The Lactic Acids

Among the oxypropionic acids with the empirical formula $C_3H_6O_3$, several are at present known to chemists.

One of these, called ethylene lactic acid, has the formula

$$CH_2(OH).CH_2.COOH.$$

This is not found in the body.‡‡

The remaining lactic acids are stereo-chemical isomerides of ethylidene lactic acid. Their formula is

$$CH_3.(CH.OH)COOH.$$

They are three in number, and the differences between them are due, according to the theory of le Bel and Van't Hoff, and as the expression stereo-chemical implies, to the space relationships of the atoms.

They differ in optical activity, and in the solubility, optical activity, and amount of crystallisation water in their zinc, calcium, lithium, and barium salts.§

The three isomerides are—

(*a*) The optically inactive acid. This is the ordinary

* Pflüger's *Archiv*, vol. lxv., p. 81.

† *Proc. Phys. Soc.*, 1904, p. ii. ; *Jour. of Phys.*, vol. xxxi.

‡‡ Small quantities of it were described in muscle extracts by Wislicenus (*Ann. d. Chem.*, 1873, vol. clxvii., p. 302) ; but this is not so ; the acid mistaken for it was acetyl lactic acid, $H_3CH(C_2H_3O_2)COOH$ (Siegfried, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1889, p. 2711).

§ Hoppe-Seyler and Araki, *Zeit. f. physiol. Chem.*, 1895, vol. xx., p. 365. Osborne, *Proc. Phys. Soc.*, 1901, p. xlix ; *Jour. of Phys.*, vol. xxvi.

fermentation lactic acid which occurs in milk when it turns sour. It has been found in small quantities in muscle.*

(*b*) Dextro-rotatory lactic acid. This is paralactic or sarcolactic acid, the lactic acid *par excellence* of muscle. It is also found in the blood, especially after muscular activity. It is found in the urine after muscular activity, during diminution of oxidation processes, in phosphorus poisoning, and after extirpation of the liver. The acidity which develops after death in many other tissues and organs is chiefly due to the same acid.

(*c*) Lævo-rotatory lactic acid. This is produced by the fermentation of cane sugar by certain kinds of bacilli, but very little is known about it at present.

In all cases where three isomerides exist, as in the present case—one optically inactive, one dextro-rotatory, and the third lævo-rotatory—it should be understood that, strictly speaking, there are only two isomerides, one dextro-, the other lævo- rotatory, the third or inactive variety being a compound of the other two. This was first shown by Pasteur in connection with racemic acid, which is optically inactive; by appropriate methods of crystallisation, it can be separated into two varieties of tartaric acid, one dextro-rotatory, the other lævo-rotatory.

Another interesting method of separating an optically inactive material into its optically active components may be described as a biological method. It consists in allowing such moulds as *Penicillium glaucum* to grow in solutions of the inactive compound; one only of its active components is destroyed by the mould, the other being left intact.

The mode of formation of lactic acid in muscles has been the subject of numerous researches; the acid has been identified as sarcolactic acid by Berzelius, du Bois Reymond, Kühne, Heidenhain, and many others. Its detection in an ethereal extract by means of Uffelmann's reaction † we have already studied (p. 6). It is found not only after death, but also on activity during life; it is doubtless one of the products the

* Heintz, *Ann. d. Chem.*, 1871, vol. clvii., p. 314.

† A dilute solution of ferric chloride and carbolic acid, which is violet, is turned yellow.

accumulation of which produces fatigue, a subject we shall have to return to when we are studying the nervous system.

In contrasting together the different kinds of muscle, Gleiss * finds that the slowly contracting red muscles of the rabbit, or the very slowly contracting muscles of the tortoise, produce acid less rapidly than ordinary voluntary muscles.

Weyl and Seitler † were the first to point out that the increase of acidity may be, at least in part, due to acid potassium phosphate produced from the alkaline phosphate by the development of new phosphoric acid from organic compounds like lecithin and nuclein. Röhmann ‡ minimises altogether the part played by lactic acid in the rise of acidity; but the more recent work of v. Fürth and of Osborne leaves no reasonable doubt that lactic acid is one if not the chief cause of the increased acidity.

With regard to the origin of sarcolactic acid, O. Nasse believed it to come from the carbohydrates in the muscle. This is, of course, the simplest view to take, and it is supported by some work of Ekunina.§ Many facts, however, do not fit in with this explanation; for instance, if the lactic acid originated from sugar and glycogen, we should expect to find the same variety of the acid that is found in milk. The view very generally held is that the acid arises from the decomposition of complex molecules, of which proteid forms a part. It is quite possible that the lactic acid may originate in both ways, and that the small quantity of fermentation lactic acid in the muscle may have a carbohydrate source.

The idea that the acid has a proteid origin was mooted by Kühne || in some of his earliest observations; he showed that not only is the acid formed during *rigor mortis*, but also during the heat coagulation of myosin. Böhm ¶ supported the proteid origin of sarcolactic acid, and his view was endorsed by Hoppe-Seyler.** Some of my own experiments, showing the develop-

* Pflüger's *Archiv*, vol. xli., p. 69.
† *Zeit. f. physiol. Chem.*, vol. vi., p. 557.
‡ Pflüger's *Archiv*, vol. lv., p. 589.
§ *Jour. f. prakt. Chem.*, N.F., vol. xx.
|| *Arch. f. Anat. u. Phys.*, 1859, p. 795.
¶ Pflüger's *Archiv*, vol. xxiii., p. 44; vol. xlvi., p. 265.
** *Phys. Chem.*, pp. 666, 667.

ment of acid during the coagulation of pure myosin,* and Latham's theoretical views † on the constitution of the proteid molecule, tend in the same direction. Araki ‡ found that the diminution of oxidation in the body, such as is produced by the inhalation of carbonic oxide, leads to the appearance of sarcolactic acid (and sometimes sugar and albumin) in the urine. This is accompanied by increase in proteid katabolism, and this again, as Hammarsten § points out, is in favour of the same view.

It will thus be seen that the bulk of authority is in favour of the theory that the lactic acid of muscle has in the main a proteid origin.

The simultaneous production of the acid, and the occurrence of *rigor mortis*, have led some investigators || to consider that the first is the cause of the second phenomenon. This implies that the acid has a non-proteid origin which is against the mass of evidence. I think we may admit that the acidity is favourable to the development of rigor, without admitting it to be the essential cause. The development of acid, and the formation of the muscle clot, have in my opinion no causal relationship between them; both are the result of changes in the myosinogen molecule.

The Nitrogenous Extractives

These are more numerous than the non-nitrogenous. In connection with some we know but little of their chemical composition, and still less of their physiological importance.

Thus *taurine* occurs in small quantity in the muscles of horses, fishes, and molluscs; *glycine* is also found in molluscan muscle. *Protic acid* is a substance of doubtful nature, also found in fishes' muscle. *Lecithin* is present in small amount,

* *Jour. of Phys.*, 1887, vol. viii., p. 134. Although these results are criticised by v. Fürth, they have been confirmed by Stewart and Sollmann, *Jour. of Phys.*, 1899, vol. xxiv., p. 450.

† *Brit. Med. Jour.*, 1886, vol. i., p. 630.

‡ *Zeit. f. physiol. Chem.*, vols. xv, xvi., xvii., and xix.

§ *Phys. Chem.*, third German edition, p. 332.

|| For instance, Catherine Schipiloff, *Centralbl. f. d. med. Wiss.*, 1882, p. 291. She found that injection of sarcolactic and other weak acids into the blood stream causes a condition of rigor in the muscles. This has been confirmed by Osborne. *loc. cit.*

but whether it is an integral component of the muscle itself, or due to the adherent nerves, is doubtful. With the lecithin are small quantities of cholesterin. *Inosinic acid* was first described by Liebig, who gave it the formula $C_{10}H_{14}N_4O_{11}$. Haiser * finds, however, that it contains phosphorus, and ascribes to it the formula $C_{10}H_{13}N_4PO_8$. It is probably related to the phosphocarnic acid to be described presently, but nothing certain is really known about it. *Carnine* is a crystalline base ($C_7H_8N_4O_3+H_2O$) originally found by Weidel in American meat extracts, but since found in the flesh of many animals. It is probably related to the members of the purine family, but again we have no certain knowledge. The list of imperfectly understood substances has been recently increased by the addition of a base called *carnosine*,† with the formula $C_9H_{14}N_4O_3$: it is probably related to arginine. The remaining members of the group are more important, and demand fuller study; to these we now pass.

Urea in Muscle

There can be but little doubt that muscular tissue, being our most abundant tissue, is the ultimate source of most of the nitrogenous waste that leaves the body as urea. The final stages in the synthesis of urea occur, as you all know, in the liver; probably what leaves the muscles is discharged as ammonia, which, uniting with carbon dioxide in the blood, forms ammonium carbamate or carbonate. The large subject of nitrogenous katabolism thus opened up is one of immense importance, and might easily occupy us for the remainder of this course of lectures; I must, however, reluctantly pass it by.

Until quite a few years ago, it was generally stated that muscle itself contains no urea, or only traces; creatine, the next substance on our list, was considered to represent it, and as we shall find, urea can be obtained from creatine in the laboratory. The statement concerning urea was partly due to imperfect

* *Monatsheft f. Chemie*, 1895, vol. xvi., p. 190.

† Gulewitsch and Amiradzbi, *Zeit. f. physiol. Chem.*, vol. xxx., p. 565.

methods of analysis. Comparatively early it was shown that urea can be obtained in fair abundance from the muscles of certain animals, for instance, arthropods.* Then many years ago Städeler and Frerichs † found that the organs, including the muscles of selachian fishes, are rich in urea. Krukenberg ‡ and Schrœder § confirmed this. In two varieties of dogfish, the mean percentage of urea in the blood was 2.61, in muscle 1.95, and in the liver 1.36. Schrœder explains this by the fact that the kidneys are sluggish in these animals. The amount of urea in these muscles, moreover, is not modified by extirpation of the liver.

By a new method, Schöndorff || has been able to satisfactorily establish the existence of a small quantity of urea in the muscles of mammals (0.07 to 0.2 per cent.).

Creatine and Creatinine

Creatine can be crystallised out by evaporating aqueous extracts of meat, from which proteids and salts have been previously removed. It can, for instance, be readily obtained from the meat extracts of commerce. You will remember the red fluid obtained from pigeon's muscle by the ether method, in which at my last lecture I showed you the spectrum of myohæmatin. I have allowed some of this to dry at room temperature in a watch glass. I will pass it round, and you will see the formation in it of some very well-formed crystals of creatine.

Creatine is closely related to another basic substance called

* Krukenberg, *Vergleich. phys. Vorträge*, 1886, p. 314.

† *Jour. f. prakt. Chem.*, 1858, vol. lxxiii., p. 48 ; vol. lxxvi., p. 58.

‡ *Loc. cit.*, p. 314.

§ *Zeit. f. physiol. Chem.*, 1890, vol. xiv., p. 576.

|| Pflüger's *Archiv*, 1895, vol. lxii., p. 1 and p. 332. Although Schöndorff's methods and results were adversely criticised by such an experienced chemist as Nencki (*Arch. f. exper. Path. Pharm.*, 1895, vol. xxxvi., p. 395), Schöndorff very successfully met these criticisms (Pflüger's *Archiv*, 1899, vol. lxxiv., p. 307), and his results have since been confirmed by Kaufmann (*Arch. de Phys. norm. et path.*, ser. 5, tome vi.) and Brunton-Blaikie (*Jour. of Phys.*, 1899, vol. xxiii., Suppl., p. 44).

creatinine, the empirical difference between the two being a molecule of water, as shown in the following equation :—

$$\underset{\text{[Creatinine.]}}{C_4H_7N_3O} + H_2O = \underset{\text{[Creatine.]}}{C_4H_9N_3O_2}.$$

According to Voit, the quantity of creatine in the voluntary muscles varies from 0.2 to 0.3 per cent. This increases during starvation. Involuntary muscle contains less than voluntary muscle.

The compound which zinc chloride forms with creatinine is generally used for isolating this substance in urine and other places where it occurs. My own experience with this method has shown it is uncertain for quantitative purposes. Perhaps this accounts for different results obtained by different observers ; thus Neubauer denies the existence of creatinine in fresh muscle altogether. Voit and others say it increases during muscular activity, whilst Nawrocki states this is not so. Much more trustworthy results are obtained by the use of G. S. Johnson's method, in which he precipitates the creatinine as a compound of mercury.* This method received the powerful recommendation of Hoppe-Seyler ; † it may be used to identify creatinine when present in small quantities; thus my pupil, P. C. Colls,‡ was able to detect it in the blood. It is quite easy to detect creatinine in meat extracts by this method. In some meat extracts examined by Johnson, and later by Kemmerich,§ the unexpected result was obtained that creatinine was more abundant than creatine. Mörner,‖ however, has proved that this is not the normal state of things ; if antiseptics are used to prevent any putrefaction, creatine is found to be the more abundant of the two.

In addition to creatine and creatinine, other basic substances, named xantho-creatinine ($C_5H_{10}N_4O$), cruso-creatinine ($C_5H_8N_4O$), amphi-creatine ($C_9H_{19}N_7O_4$), and pseudo-xanthine ($C_4H_5N_5O$), are stated by Gautier ¶ to

* *Proc. Roy. Soc.*, vol. xlii., pp. 365, 493 ; vol. l., p. 28.
† *Handb. d. phys. chem. Anal.*, 1893, seventh edition, p. 142.
‡ *Jour. of Phys.*, 1896, vol. xx., p. 107.
§ *Zeit. f. physiol. Chem.*, 1894, vol. xviii., p. 409.
‖ Du Bois' *Archiv*, 1898, p. 266.
Maly's *Jahresbericht*, vol. xxii., p. 335.

occur in small quantities. In fishes' muscle Thesen * describes an iso-creatinine. All these are, however, at present mostly in the region of the unknown.

The main physiological interest of creatine and creatinine centres around their relationship to nitrogenous metabolism. By chemical means creatine can be decomposed into sarcosine or methyl amino-acetic acid and urea, as shown in the following equation :—

$$\underset{\text{[Creatine.]}}{C_4H_9N_3O_2} + H_2O = \underset{\text{[Sarcosine.]}}{NH.CH_3.CH_2.COOH} + \underset{\text{[Urea.]}}{CON_2H_4}.$$

It is quite possible that this may occur in the body, though we are not always justified in concluding that the decompositions of the laboratory are comparable to those taking place during metabolism. We are justified in assuming this sceptical attitude because injection of creatine into the blood stream of an animal leads to the increase in the urine, not of urea but of creatinine.

Most of the creatinine of the urine is derived from the creatine contained in the meat of the food. There is, however, a small amount of creatinine in the urine, even during starvation, which appears to represent a small percentage of creatine from the muscles. The fate of the muscular creatine is one of the many puzzling problems in relation to the general question of nitrogenous metabolism which I promised to avoid. The small amount of creatinine in the urine apart from that which originates from the creatine of the food will not account for it all. I think we may fairly conclude that the liver forms urea from simple ammonium compounds, and so we must assume that the most of the creatine is broken up into ammonia before it leaves the muscles.

Uric Acid, Xanthine, and Hypoxanthine

These three substances are found only in small quantities; the numbers given are xanthine, 0.0026 ; hypoxanthine, 0.022-0.026 per cent.; uric acid, traces. Uric acid is, however, more abundant in reptilian muscle.

These three substances are important members of the

* *Zeit. f. physiol. Chem.*, vol. xxiv., p. 1.

purine family, and their names in this connection are as follows:—

Hypoxanthine	$C_5H_4N_4O$. Oxypurine.
Xanthine	$C_5H_4N_4O_2$ Dioxypurine.
Uric Acid	$C_5H_4N_4O_3$ Trioxypurine.

Their formulæ sufficiently indicate their close connection.

The interest of these substances is derived in the first instance from their origin; they are the products of nuclein metabolism. In the second place, they have a pathological interest, and they would lead us, if we were not careful to keep to the main track, into the pathological side-issue of the causation of gout and allied disorders. I will be content to state the following main conclusions arrived at in connection with this question.

1. The synthetic formation of uric acid from ammonia and lactic acid, which is so important in birds, occurs in mammals to a slight extent only.

2. The most important origin of uric acid in mammals is by decomposition of nuclein, and oxidation of the purine bases so liberated.

3. Certain forms of diet increase uric acid formation by leading to an increase of nuclear metabolism. This is indicated in many cases by increase of the leucocytes, and, consequently, increase in the metabolism of their nuclei. Although special attention has been directed to the nuclei of leucocytes because they are so readily examined during life, it must be remembered that nuclein metabolism in all cells may contribute to uric acid formation.

4. Certain forms of diet increase the uric acid in the urine and body generally, because they themselves contain nuclein or nuclein derivatives in abundance. Sweetbread and liver are typical instances of this sort of food.

5. Uric acid which comes directly from nuclein or purine substances in the food may be conveniently spoken of as *exogenous:* that which arises from metabolism is termed *endogenous* (Burian and Schur).

6. Meat diet causes an increase in uric acid, partly because

it stimulates cellular (*e.g.* leucocytic) activity, but mainly because it increases the exogenous uric acid from the purine bases, especially the hypoxanthine which it contains.

Carnic Acid

Fleischsäure (Carnic acid) is the name given by Siegfried* to a constituent of muscle, to the discovery of which he attributed great importance. He first prepared it from muscle extracts by means of ferric chloride ; the compound so obtained is called carniferrin ; this contains phosphorus as well as iron. By means of baryta water, carnic acid ($C_{10}H_{15}N_3O_5$) was separated out from it. In muscle, carnic acid is combined with phosphorus to form phospho-carnic acid or nucleon. The first startling announcement made by Siegfried was that carnic acid is identical with Kühne's antipeptone. Balke,† working under Siegfried's supervision, subsequently prepared compounds and derivatives of this material, which need hardly concern us considering the subsequent history of the investigation. On decomposition it yields succinic acid, sarcolactic acid, a reducing carbohydrate, and other substances. This was followed by work by Krüger,‡ and what we may call a second startling announcement, namely, that on hydrolysis it gives off carbon dioxide. He, therefore, looked upon it as the material in muscle which during muscular activity gives off carbon dioxide without the using up of oxygen ; in fact, it was the first substance separated out from muscle by chemists which fulfilled the characters ascribed by Hermann to his hypothetical inogen.

Krüger,§ however, in his subsequent work, discovered that various preparations of nucleon gave very different percentages of nitrogen, and Siegfried himself was compelled to admit that the varying relationship of nitrogen to phosphorus shows that the composition of nucleon is not so certain as he had at first supposed. About the same time, also, Kutscher‖ proved that

* *Ber. d. deutsch. chem. Gesellsch.*, 1894, vol. xxviii., p. 2762 ; *Zeit. f. physiol. Chem.*, vol. xxi., p. 360.

† *Zeit. f. physiol. Chem.*, 1896, vol. xxi., p. 380 ; vol. xxii., p. 248.

‡ *Ibid.*, vol. xxii., p. 95.

§ *Ibid.*, vol. xxviii., p. 530.

‖ *Ibid.*, vol. xxvi., p. 110.

antipeptone, although it is not a true peptone, is nevertheless not a chemical unit (carnic acid), as alleged by Siegfried, but a mixture of various substances of which he separated out aspartic acid, and the hexone bases histidine and arginine.

Siegfried's work has the merit that it was one of the earliest to shake the foundations on which Kühne's theory of anti- and hemi- products of digestive proteolysis was built. He has also drawn attention to certain phosphorised constituents of muscle which had hitherto escaped attention. He has, however, failed to prove that nucleon is a chemical individual, and also that it has the importance and interest which he at first considered it to possess.

The Inorganic Salts of Muscle

The total ash varies from 1 to 1.5 per cent. The following analyses are by Bunge* :—

	In parts per 1000.	
	I.	II.
K_2O	4.654	4.160
Na_2O	0.770	0.811
CaO	0.086	0.072
MgO	0.412	0.381
Fe_2O_3	0.057	...
P_2O_5	4.644	4.580
Cl.	0.672	0.700
SO_3	...	0.100

J. Katz† gives the following numbers from the examination of the flesh of a large number of animals; they represent the minimum and maximum in 1000 parts of fresh flesh.

K	2.4 to 4.6
Na	0.3 to 1.5
Fe	0.04 to 0.25
Ca	0.02 to 0.39
Mg	0.18 to 0.37
P (from phosphates)	1.22 to 2.04
(from lecithin)	0.13 to 0.48
(from nuclein)	0.09 to 0.32
Cl	0.32 to 0.8

* *Zeit. f. physiol. Chem.*, vol. ix., p. 60.

† Pflüger's *Archiv*, 1896, vol. lxiii., p. 1.

We at once note the predominance of potash * among the bases, and of phosphoric acid among the acids.

I will conclude with a passing allusion to the importance of mineral constituents in the causation of muscular contraction. Many years ago, Ringer showed that contractile tissues continue to manifest their activity in pure solutions of salts corresponding in proportion to those found in the blood. This question has more recently been taken up in America by Howell at Baltimore, and Loeb at Chicago and California. Since the introduction of the ionic theory, these results of Ringer's have been interpreted as ionic action. Contractile tissues will not contract in pure solutions of non-electrolytes (sugar, urea, albumin). But different contractile tissues differ in the nature of the ions which are most favourable stimuli. Loeb divides them into three classes: (1) These which produce such contractions; of these the most efficacious is Na. (2) Those which retard or inhibit rhythmical contractions; for instance, Ca and K. (3) Those which act catalytically; that is, they accelerate the action of Na, though they do not themselves produce rhythmical contractions directly; for instance, H and OH. In spite of the antagonistic effect of Ca, a certain minimal amount of it must be present if contractions are to continue for any length of time. We have already seen that the influence of calcium, which is so important in blood-clotting and milk-curdling, is a doubtful factor in the production of muscle-clotting or *rigor mortis*. There is, however, no doubt as to its necessity in muscular contraction. Ions produce contraction because they affect either the physical condition of the colloidal substances (proteid, etc.), in protoplasm, or the rapidity of chemical processes.

* Prof. A. B. Macallum has recently introduced a micro-chemical test for potassium in tissues. It consists in adding to the fresh tissue a mixture of the nitrites of sodium and cobalt; an abundant precipitate of yellow crystals is the result. If the amount of potassium is small, there is only a yellow colour; this is turned black (cobalt sulphide) on the addition of ammonium sulphide. The black particles seen form a very delicate test. In a muscular fibre the potassium is limited to the dark bands. His paper will shortly appear in the *Journal of Physiology*.

LECTURE V

CHEMICAL CHANGES ACCOMPANYING THE CONTRACTION OF MUSCLE. CHEMISTRY OF TENDON

We have now to attempt an answer to what is, after all, the most important question in relation to muscular activity, namely, what are the chemical changes which accompany its contraction. There is no doubt that it is the chemical change which underlies the other manifestations of muscular activity; the heat produced, and the electrical current of action being secondary to this. In the transformation of the energy of chemical affinity, some reappears as heat, and a variable but comparatively small fraction as muscular work.

It is in order to answer this question that up to this point we have been gathering materials. We are somewhat in the position of an engineer who has been collecting the individual parts of a machine, and learning the uses of each before he proceeds to investigate the way in which the machine works as a whole. It is just here we have to confess the imperfection of our knowledge; we have, it is true, a number of facts to rely upon; we have noted, however, in going along, how many more facts we ought to have before we can found trustworthy and workable theories.

While a muscle is at rest, we do not mean it is absolutely inactive; we know, for instance, that it possesses that small amount of contraction which is technically known as "tonus." There is also what we may call "chemical tonus"; the evidence of this is that the blood leaving muscles which are not contracting is nevertheless venous, and heat production is occurring in muscles which are in repose. In all probability,

the chemical changes that occur during contraction are similar in kind to those which occur during so-called rest; there is a sudden exaggeration of the normal "chemical tonus" of the tissue, and an explosive liberation of energy.

The first trustworthy observations in this connection were made by Helmholtz; he showed that during contraction the extractives soluble in alcohol increase, and those soluble in water decrease. This is chiefly explicable by the disappearance of glycogen, and the appearance of sugar and lactic acid. Research has shown that on contraction, proteid katabolism is somewhat increased; still in a normally nourished muscle the main work falls on the non-nitrogenous part of the muscle, as is shown by the immediate increase in the amount of carbon dioxide which leaves it. (See Lecture IV).

In a general way we may speak of the essential chemical process as one of oxidation, in that oxidised products are discharged; but the oxidation is not of that simple kind which occurs when oxygen combines with the combustible material in a candle. Oxygen in muscle, as in other tissues, is not only of importance for the formation of katabolic products, but is of even greater use in anabolism; it assists in the preliminary building up of complex materials within the living molecules. Hermann's celebrated inogen theory was the outcome of such a conception; there is no oxygen among the gases which he extracted from muscle, and a muscle will continue to contract and give off carbon dioxide in an atmosphere free from oxygen. The oxygen used in the formation of this carbonic acid must therefore have been held in some compound within the tissue, and this hypothetical body was termed *inogen*. The theory in all its details no longer stands; yet it embodies the undoubted fact that the *rôle* of oxygen is more in the constructive than in the destructive phase of metabolism. The term oxygenation better expresses this than oxidation. The difficulties surrounding the question have led some physiologists to postulate the existence of *oxidases*, or oxygen carrying ferments. I regard it as very questionable whether they are really enzymes, but if such materials exist, it appears probable that their usefulness is not in promoting oxidation, a katabolic change, but rather what

we have just called oxygenation, that is the building of the oxygen into the living molecules.

Hermann's theory of muscular contraction further assumes that the change is similar in kind to that which occurs on death, though less in degree. On death he considered that inogen is split into carbon dioxide, sarcolactic acid, and myosin. He thus throws in his lot with those who hold that sarcolactic acid comes from a proteid complex and not from carbohydrate. We have, however, no proof that any formation of a clot of myosin occurs in the contraction of living muscle; in fact, the observations on the extensibility of living contracted muscle, as compared with that of rigored muscles, tend in exactly the opposite direction.

Hermann's theory put in another way, that living contracted muscle is muscle on the road to death, could only be proved by finding an exact parallelism in all details; the table I am going to give you immediately shows in how many details the analogy breaks down. The theory is interesting from an historical point of view, and it also serves as a useful peg for a lecturer to fix the students' attention when speaking of the similarities in the two cases.

Here is the table I just mentioned.

Dead Muscle in *Rigor Mortis*.	Living Muscle in Contraction.
1. Muscle shortened and opaque.	1. Muscle shortened, but not opaque.
2. Carbon dioxide given off.	2. Carbon dioxide given off.
3. Sarcolactic acid formed.	3. Sarcolactic acid formed.
4. Heat evolved at onset of rigor.	4. Heat evolved on contraction.
5. Muscle becomes electro-positive to living muscle.	5. Muscle becomes electro-positive at commencement of contraction
6. Muscle plasma clots.	6. Muscle plasma does not clot.
7. Extensibility is diminished.	7. Extensibility is increased.
8. Rigor is hindered by dextrose.	8. Contraction is favoured by dextrose.
9. Whether calcium is essential is uncertain.	9. Calcium is essential.

I think you will agree with me that our knowledge on this question is unsatisfactory. It is so fragmentary; and even if I could piece together all the other fragments of our present knowledge which I have omitted, we should still be without the means

of producing a perfect picture. I will only trouble you with one more fragment, and this relates to the formation of reducing substances. Resting muscle oxidises pyrogallic acid; tetanised muscle does not. A solution of nitrites passed through contracting muscle is changed into one of nitrates, and the colour of solutions of indigo sulphate is altered in the same way as by reducing agents.* A. Schmidt † arrived at the same conclusion from the examination of the venous blood of tetanised muscle; but what the reducing substances are that are produced is quite unknown.

Chemistry of Tendon

It is rather a sudden jump to pass from such an eminently living tissue as muscle to a comparatively passive material like its tendon. The anatomical proximity of the two is my excuse for doing so.

The chemistry of the connective tissues has always possessed a great attraction for me, and I was originally led to take it up in connection with the question of myxœdema. In this disease there is not only the atrophy of the proper substance of the thyroid gland, and various nervous symptoms which I need not go into, but also a swollen condition of the body which led to the adoption of the name myxœdema. This name will probably not be altered now, but it is nevertheless a misnomer. The idea in Ord's mind that the swollen appearance of the subcutaneous tissues is due to excess of mucin has been abundantly disproved. There is only a slight increase of mucin in an early stage of the overgrowth of these tissues; new connective tissues, as in the fœtus and child, always contain a relatively large amount of intercellular or ground substance, of which an important constituent is mucin. In later stages, when development of fibres or deposition of fat takes place, this relative increase of mucin disappears.

My former pupil, Dr R. A. Young, took up at my suggestion a number of other questions in connection with the connective

* Grützner, Pflüger's *Archiv*, vol. vii., p. 255; Gscheidlen, *ibid.*, vol. viii., p. 506.

† *Sitzungsber d. Akad. d. Wissensch.*, Wien, vol. xx.

tissues, but of recent years the centre of work on these points has shifted across the Atlantic, and Dr William J. Gies is the investigator who has now made the subject his own. His numerous papers will be mainly found in the *American Journal of Physiology*.

A tendon, and we must restrict ourselves to this, contains about 63 per cent. of water. The most abundant solid is collagen, the material of which the white fibres are composed. It is so named because it is the mother substance of gelatin, into which it is easily converted by boiling. The other histological elements are the tendon cells and the ground substance. This latter material is generally (on the strength of the older observations of Rollett and Loebisch) spoken of as muco-albuminous, an adjective which recent work has shown to be correct. The total amount of mucin is generally under 1 per cent., and the amount of albumin is even less. Small quantities of extractives (including creatine) and inorganic salts complete the list.

The ground substance of connective tissue was investigated by Young in the vitreous humour of the eye, and the Whartonian jelly of the umbilical cord, because in those situations fibres and cells are at a minimum. Mucin is a term which covers a number of substances, all soluble in dilute alkali, precipitable by acetic acid, and in constitution consisting of proteid combined with a carbohydrate radicle. The difference in proportion between the proteid and carbohydrate components probably accounts for the differences between the members of the mucin group. The carbohydrate material was at one time called "animal gum," after Landwehr; the reducing substance obtained by boiling mucin with mineral acid is not sugar, but an amino-derivative, glucosamine ($C_6H_{11}O_5.NH_2$), in many cases at any rate.

The gluco-proteids of connective tissue are now generally called mucoids, the term mucin being restricted to gluco-proteids of cellular origin, for instance in the goblet cells of columnar epithelium. Though Gies, from slight discrepancies in ultimate analyses, suspects that more than one member of the gluco-proteid group is present in connective tissue, he has shown that the principal mucoid in tendon (tendo-mucoid) is

the same material as that which can be separated out from other forms of connective tissue (chondro-mucoid, osseo-mucoid, etc.).

Young and Gies agree that the proteid matter (other than the mucoid) contained in the ground substance can be separated into an albumin and a globulin. But the amount of each is so small that their characters have not been fully worked out; moreover, it is quite probable that in part these substances are derived from adherent lymph. Their temperature of heat coagulation is differently given by the two observers, and this again is what one expects in experiments in which dilute solutions of proteid are employed. Brodie and Richardson, in their work on heat rigor of muscle, found that tendon also shortens very considerably at a certain temperature, namely, 63° C., and this temperature is very constant, but we cannot at present say which proteid constituent of tendon is responsible for this.

Another question which we must answer before concluding is, whether tendon contains reticulin. It is first necessary to explain what reticulin is. The fibres of reticular or retiform tissue (found in such situations as lymphatic glands, or the corium of many mucous membranes) are anatomically continuous with those of areolar tissue, and are not distinguishable from them on microscopic examination. It would therefore have been remarkable, if they were demonstrated to be chemically different from ordinary white fibres. The statement that this is so was, however, made by Mall.* At first he thought they were made of elastin, but subsequently, finding this was not so, he considered that they were made of something else, but certainly not of collagen, because no gelatin could be obtained from them. This error was pointed out by R. A. Young;† gelatin is obtained from reticular fibres with great ease, and this observation was subsequently confirmed by Siegfried.‡ Siegfried, however, stated that, in addition to col-

* *Anat. Anzeiger*, 1888, vol. iii., No. 14; *Abhandl. d. math. phys. Cl. d. k. Sächs. Gesellsch. d. Wissensch.*, 1887, vol. xiv., No. 3; 1891, vol. xvii., No. 4.

† *Jour. of Phys.*, 1892, vol. xiii., p. 332.

‡ *Ueber die chemischen Eigenschaften des reticulirten Gewebes.* Habilitationschrift, Leipzig, 1892.

lagen, the reticular fibres contain something special, and separated from them not only gelatin, but another chemical substance specially resistant to the action of reagents, and to this he gave the name of reticulin. If such a chemical substance does exist, the point is by no means proved that reticular fibres are different from white connective-tissue fibres; it is at least equally possible that reticulin may be present in all white connective-tissue fibres, and I therefore suggested to Miss Tebb that she should look for it in a typical form of connective tissue, namely, tendon.

Siegfried's work had been so highly spoken of, and his conclusions accepted so unhesitatingly by Dr Gamgee,* that you may well imagine that when Miss Tebb was unable to find a trace of reticulin in tendon, my disappointment was very great. But, instead of stopping here, this failure to obtain reticulin from tendon led us next to repeat Siegfried's experiments on the tissue with which he had himself worked, namely, the mucous membrane of the intestine. I knew from previous experience that the prolonged action of alcohol on collagen renders it very difficult to convert into gelatin. Miss Tebb showed the same to be true for ether, though in a less degree. Neither alcohol nor ether, however, have any appreciable effect of this kind on gelatin. Both of these reagents were used by Siegfried for prolonged periods to extract the fat from the mucous membrane. It was therefore quite on the cards that reticulin was merely an artifact, and the conclusion at which Miss Tebb arrived may be best expressed in her own words:—

"My main result is that reticulin does not exist either in ordinary white fibrous tissue (tendon), or in the reticular tissue of the intestinal mucous membrane. Both consist of fibres which are chemically and histologically identical; the main material of which they are composed is the gelatin-yielding substance called collagen.

"I regard Siegfried's reticulin merely as collagen which has been 'coagulated' by the reagents he employed (especially alcohol and ether), *plus* proteid and nuclein residues of cells. After treatment with these reagents, the conversion into gelatin is much more difficult, not only in the case of a finely stranded

* *Phys. Chem.*, 1893, vol. ii., p. 402.

tissue like reticular tissue, but even in a dense material like tendon."

I can vouch for the accuracy and care with which Miss Tebb carried out the investigation, and it was most curious to see, as I watched the progress of the work, how erroneous even on minor points Siegfried's observations had been. The two new substances, carnic acid and reticulin, with which Siegfried's name is linked, have thus both met with an unfortunate end.*

We have now completed our study of the first subject of the course, and the remaining lectures I have to deliver will deal with some of the biochemical aspects of nerve physiology.

* Siegfried published a polemical reply to Miss Tebb in the *Jour. of Phys.*, 1902, vol. xxviii., p. 319. He does not, however, shake in the least the conclusions she arrived at.

LECTURE VI

THE CHEMICAL COMPOSITION OF NERVOUS TISSUES

THE nervous system has always a great fascination for physiologists and physicians. It is the ruling system of the body, regulating and controlling the other processes that occur there. It is, moreover, the seat of mental phenomena, and so its investigation appeals to all those who have sought to unravel the so-called mysteries of thought and reason. But neither the scalpel, the microscope, or the test-tube have as yet succeeded in discovering the mind. Still, even in our test-tubes we shall find something that is of interest, and I hope also of practical importance. We shall first deal with the physiological, and later with the pathological, side of the subject. We shall therefore start with the general chemical composition of nervous tissues.

Relation of Water and Solids

The first general impression one derives from a glance through any of the analytical tables in the text-books, is the great preponderance of water in most of our so-called solid tissues. Even bone contains nearly 50 per cent. of water, and muscle, as we have already seen, contains 75 per cent. or more.

The nervous system is no exception to this rule. The amount of water varies; it is present in larger amount in early than in adult life, in grey than in white matter, in the brain than in the spinal cord, in the spinal cord than in nerves.

This is illustrated by the following tables:—

*Influence of Age on the Percentage of Water in the Brain Tissue.**

	White Matter.	Grey Matter.
In fœtus	87	92
Age 20-30	69	83
Age 70-90	72	84

Percentage of Water in Different Parts of the Nervous System of the Adult.†

Grey matter of brain	81-86
White matter of brain	68-72
Brain as a whole	81
Spinal cord	68-76
Nerves	57-64

Percentage of Water in Different Parts of the Nervous System.‡

Grey matter of cerebrum	83.4
White matter of cerebrum	69.9
Cerebellum	79.8
Spinal cord as a whole	71.6
Cervical cord	72.5
Dorsal cord	69.7
Lumbar cord	72.6
Sciatic nerves	65.3

We thus see that water is most abundant in the grey matter, or in those regions of the nervous system where the proportion of grey matter is greatest. It cannot fail to be a striking fact that the grey matter, the region which is most active and most important, contains somewhat less than 17 per cent. of solid materials.

Specific Gravity

The question of the amount of water is closely related to that of specific gravity. The specific gravity of the brain has been the subject of researches by Bastian, Bischof, Danilewski, and others, but I only wish here to dwell upon one aspect of the

* Weisbach, *Hofmann's Lehrbuch d. Zoochemie*, Wien, 1876, p. 121.

† Table compiled from a number of analyses made by others.

‡ This table gives the averages of a large number of experiments made with the organs of human beings, monkeys, dogs, and cats, by myself (*Jour. of Phys.*, 1893, vol. xv., p. 90).

question which was brought prominently before the medical profession, in an address on "Sex in Education," by Sir James Crichton Browne,* some years ago. Among the differences between the brains of men and women, Sir James stated that he had found that the specific gravity of the female brain is less than that of the male brain. It was, however, pointed out in the correspondence that followed the publication of the address, that this generalisation rested on very few observations. I have accordingly thought it advisable to examine the brains in a large number of cases. This has been carried out in my laboratory by R. H. Gompertz, B.Sc.† All I shall do here is to mention his main conclusion. He finds that in adult men and women, who suffered from no brain disease, that there are fairly wide variations in both sexes, but that the average (1.035) is identical in male and female, and that there is no foundation for the belief that the variations constitute a sexual difference.

I may point out that a low specific gravity of the brain does not necessarily imply a poorer quality, for the part of the brain which is most important and most active—the grey matter—has a lower specific gravity than the white matter.

If, for instance, Sir James Crichton Browne had satisfactorily established his contention that a woman's brain is of lower specific gravity than a man's, the conclusion might have been drawn that the female brain is richer in grey matter, and the ladies might quite fairly have argued that what they lacked in quantity was made up in quality. The question, however, is not so simple as this, for, as Dr Bastian pointed out to me, an increase of association fibres which doubtless underlies intellectual superiority would mean an increase of white matter, and lead to a raising of the specific gravity. Boileau ‡ drew attention, in the examination he made of the brain of a highly gifted man, not only to its great weight, but also to its *high* specific gravity. A great many more similar cases will have to be recorded, before any general rule can be laid down on the subject.

* *Brit. Med. Jour.*, 1892, vol. i., p. 949.
† *Jour. of Phys.*, 1902, vol. xxvii., p. 459.
‡ *Lancet*, 1882, vol. ii., p. 485.

Solids of Nervous Tissues

But coming now to the more important subject of the solids, we find it is possible to divide them into the following classes :—

(*a*) Proteids. These comprise a very considerable percentage of the solids, especially in the grey matter (over 50 per cent.).

(*b*) Nuclein. From the nuclei of the cells.

(*c*) Neuro-keratin. From the supporting framework (neuroglia).

(*d*) Phosphorised fats (protagon, lecithin, etc.).

(*e*) Cerebrins or cerebrosides.

(*f*) Cholesterin.

(*g*) Extractives. Small quantities of numerous organic substances, of which creatine, xanthine, hypoxanthine, inosite, lactic acid, uric acid, and urea have been identified.

(*h*) Gelatin. From the adherent connective tissue.

(*i*) Inorganic salts. Of these, alkaline phosphates and chlorides are the most abundant, but the total ash is only about 1 per cent.*

The following table gives some typical quantitative analyses which have been made of the proportion in which the principal solids occur in different nervous structures :—

Portion of Nervous System.	Proteids.	Lecithin.	Cholesterin and fat.	Cerebrin.	Neuro-keratin.	Other organic matters.	Salts.
Grey matter of ox brain (Petrowsky)	55.37	17.24	18.68	0.53	6.71		1.45
White matter of ox brain (*ibid.*) . .	24.72	9.90	51.9	9 55	3.34		0.57
Spinal cord (Moleschott)	23.8	75.1					1.1
Human sciatic nerve (Josephine Chevalier)	36.8	32.57	12.22	11.30	3.07	4.0	...

* It appears desirable that fresh investigations on the ash of nervous tissues should be undertaken. Macallum has shown by his micro-chemical test (see p. 48) that potassium is absent from the nerve-cell and the axon. It is present in the interstitial tissue, and occurs in curious patches within the neurilemmal sheath. Another important generalisation of his work is that potassium is absent from the nuclei of all animal and vegetable cells.

We are thus provided at the outset with a very long list of substances to consider, but happily for you, though no doubt unhappily for science, we know comparatively little of many of these mentioned, or at least we are unacquainted with their physiological significance. In connection with the group of extractives, for instance, we can surmise that they are mostly waste products, as they are elsewhere. In connection with cholesterin, the large amount present of this remarkable monatomic alcohol cannot be devoid of importance, but at present we cannot even conjecture what that importance consists in. Of the substances mentioned, those which will require further notice are the proteids, the phosphorised fats, and the cerebrosides.

Proteids of Nervous Tissues

The large amount of proteid matter, next to the high percentage of water, is the most striking fact in the preceding table. The highest percentage is found where one would expect it, namely, in the grey matter, where the protoplasmic structures, the nerve-cells, occur.

The following table is a compilation from my own analyses :—

	Water.	Solids.	Percentage of proteid in solids.
Grey matter of cerebrum . . .	83.467	16.533	51
White „ „ . . .	69.912	30.088	33
Cerebellum	79.809	20.191	42
Spinal cord as a whole . . .	71.641	28.359	31
Cervical cord	72.529	27.471	31
Dorsal cord	69.755	30.245	28
Lumbar cord	72.639	27.361	33
Sciatic nerves	61.316	38.684	29

This table illustrates the fact that the amount of grey matter, of water, and the percentage of proteid in the solids, vary directly the one with the others. This is very well seen in the different regions of the spinal cord. The percentage of proteid matter in the white matter of the brain is a little higher than in the spinal

cord. This is the only exception to the general rule, and perhaps may be explained by the high percentage of neuro-keratin in white matter.*

The earliest to study the nature of the proteids was Petrowsky.† He investigated the question previous to our modern ideas concerning proteids, and described them as consisting of a globulin somewhat resembling myosin, and an albumin especially abundant in grey matter. Baumstark,‡ in a more recent research, speaks of the chief proteid matter in nervous tissue as resembling casein. There is a certain amount of truth in this, for it is a nucleo-proteid. A few years later I took up the matter, and the following were my chief conclusions.§

The proteids present are three in number. They differ in temperature of heat coagulation, in the readiness with which they are precipitated by neutral salts, and by acetic acid; one of them contains phosphorus and is a nucleo-proteid, so differing from the other two, which are globulins. The most important characters of these proteids are the following:—

(*a*) This proteid is a globulin; it may conveniently be termed neuro-globulin *a*. It is coagulated by heat at the low temperature of 47°, and is analogous to similar globulins which are found in all cellular structures, such as cell-globulin of lymph-cells, para-myosinogen of muscle, hepato-globulin *a* of the liver, and kidney globulin. Indeed, this proteid seems to be as constant a constituent of protoplasmic structures as the nucleo-proteids are.

It is precipitated by a comparatively small percentage of such neutral salts as magnesium sulphate. It is not precipitated by weak acetic acid. It contains no phosphorus in its molecule.

In view of the subject of hyperpyrexia, a pathological problem we shall be considering later, I would ask you to make a mental note of the low coagulation temperature of this proteid.

* The percentage of neuro-keratin is in grey matter, 0.3; in white matter, 2.2 to 2.9; and in nerve, 0.3 to 0.6 per cent. (Kühne and Chittenden, *Zeit f. Biol.*, vol. xxvi., p. 291).

† Pflüger's *Archiv*, vol. vii., p. 367.

‡ *Zeit. f. physiol. Chem.*, vol. ix., p. 145.

§ *Jour. of Phys.*, vol. xv., p. 106.

(*b*) This proteid is a nucleo-proteid. It can be readily prepared from nervous tissues by making a saline extract, but under these circumstances it is mixed with the other proteids. It may, however, be prepared in large quantities by precipitating an aqueous extract of brain by weak acetic acid. The supply obtainable from white matter is small.

It is coagulated by heat at 56 to 60°. Like globulins, it is precipitable by saturating its solutions with neutral salts; but more salt is necessary than in the case of neuro-globulin *α*.

It contains 0.5 per cent. of phosphorus.

After subjection to gastric digestion, an unsoluble residue of nuclein remains behind.

Dissolved in dilute sodium carbonate and injected into the vascular system of rabbits, it causes, like other nucleo-proteids, extensive intravascular coagulation.

(*c*) This proteid is a globulin. It may conveniently be called neuro-globulin *β*, and is closely analogous to the hepato-globulin *β* of liver cells. It is coagulated by heat at 70 to 75° C.; it is precipitable by neutral salts, but requires complete saturation with magnesium sulphate to precipitate it entirely. It is not precipitable by weak acetic acid like the nucleo-proteid just described, and contains no phosphorus in its molecule.

The only other research with which I am acquainted on the proteids of nervous tissues is one by P. A. Levene.* He has particularly directed his attention to the nucleo-proteid of the brain, and although he separated it out from the organ by a method different from that which I employed, he was evidently dealing with the same substance; the amount of phosphorus being 0.5 per cent., which is the same number I obtained. The purine bases obtainable from cerebro-nucleo-proteid are guanine, adenine, small quantities of xanthine, but no hypoxanthine.

The Phosphorised Fats

The best known member of this group is lecithin, and the compound of lecithin with cerebrin which is called protagon. But there are several lecithins differing in the kind of fatty acid

* *Archives of Neurology and Psychopathology*, 1899, vol. vii., p. 14.

they contain, and other substances of similar nature like kephalin which has recently been the subject of a research by Waldemar Koch. Koch suggests the name of the *Lecithans* for the whole group, in place of *Phosphatides* introduced by Thudichum. I myself prefer the expression *phosphorised fats*, which, although it is a little longer, is quite free from ambiguity.

It was in the year 1865 that Liebreich * separated from the brain the material he termed *protagon;* he further found that when decomposed by baryta-water it yields two acids—stearic acid and glycero-phosphoric acid—and a base called choline.

Hoppe-Seyler, and Diaconow † working under Hoppe-Seyler's direction, denied the existence of this substance protagon, and considered that it was a mere mechanical mixture of the phosphorised fat called *lecithin*, with a nitrogenous non-phosphorised substance called *cerebrin.* Lecithin yields the same three decomposition products that were obtained from protagon by Liebreich. Diaconow's elementary analyses were, however, far from convincing.

The subject in this country was taken up by Gamgee and Blankenhorn ; ‡ and the result of their work has been that Liebreich's discovery has been fully verified. They showed that protagon is a perfectly definite crystalline substance of constant elementary composition. They also showed that even prolonged treatment with alcohol and ether will not extract lecithin from protagon, as alleged by Diaconow. When protagon is digested with alkalis, it yields cerebrin or cerebrins, and the decomposition products of lecithin.

This work has been confirmed by Baumstark,§ Ruppel,‖ and Kossel and Freytag.¶

Protagon is prepared by digesting brain with alcohol at 45° C., the extract is filtered while warm, and then cooled to 0° C. Protagon crystals mixed with cholesterin are thus deposited.

* Liebreich, *Annalen der Chem. u. Pharm.*, vol. cxxxiv., p. 29.
† Diaconow, *Centralbl. f. d. med. Wissensch.*, 1868, p. 97.
‡ Gamgee and Blankenhorn, *Jour. of Phys.*, vol. ii., p. 113.
§ Baumstark, *Zeit. f. physiol. Chem.*, vol. ix., p. 329.
‖ *Zeit. f. Biol.*, vol. xxxi., p. 86.
¶ *Zeit. f. physiol. Chem.*, vol. xvii., p. 431.

The cholesterin is dissolved out by ether. The protagon is then collected, redissolved in warm alcohol, and allowed to recrystallise on cooling. Its empirical formula was calculated by Gamgee and Blankenhorn from their analytical results to be $C_{160}H_{308}N_5PO_{35}$. But this formula will require revision, as both Kossel and Ruppel have shown that the molecule contains a small amount of sulphur which had been overlooked by the earlier observers.

The doubt as to the chemical individuality of protagon has arisen again in recent years. Kossel and Freytag in their work assumed the existence of several protagons, differing in the nature of the lecithin, and also of the cerebrin they contain. Lesem and Gies * doubt whether protagon is a chemical unit, but regard it as a mixture.

This is a view which I cannot consider they have proved; protagon is a definite crystalline material; it has been analysed with remarkably concordant results by numerous investigators. Moreover, Cramer † has within the last few months prepared protagon by a slightly different method, and shows that its composition is constant. Allowing for the sulphur, he calculates its empirical formula to be $C_{320}H_{616}N_{10}P_2SO_{68}$. Its molecular weight would therefore be 5778.

Although I differ from Lesem and Gies in one of their conclusions, I agree with another which they put forward, namely, that protagon (whether it is a unit or not), does not contain the bulk of the phosphorised organic substances of nervous material. There is no doubt that the bulk of the phosphorus is contained within materials of smaller molecular weight, such as lecithin and kephalin. The amount of organic phosphorus is so great that it would be quite impossible for much of it to be contained within a substance of such high molecular weight as protagon.

Lecithin

This may be taken as a type of the phosphorised fats; it is certainly an abundant constituent of nervous tissues, and the

* *American Jour. of Phys.*, 1902, vol. viii., p. 183.

† *Jour. of Phys.*, 1904, vol. xxxi., p. 30.

one concerning which we have most knowledge. It is also a constituent of the protoplasm of all cells. When it is decomposed, either in the laboratory or in the body, it breaks up into three substances, as shown in the following equation :—

$$\underset{\text{[lecithin]}}{C_{44}H_{90}NPO_9} + 3H_2O = 2\underset{\text{[stearic acid]}}{C_{18}H_{36}O_2} + \underset{\substack{\text{[glycero-}\\ \text{phosphoric acid]}}}{C_3H_9PO_6} + \underset{\text{[choline]}}{C_5H_{15}NO_2}$$

To give this substance its chemical name, we must call it choline-distearyl-glycero-phosphoric acid. The choline radicle is united to the acid by means of the oxygen of the hydroxyl ; it is therefore not a salt but an ether-like combination, thus :—

$$\begin{array}{l} CH_2O - C_{17}H_{35}CO \\ CHO - C_{17}H_{35}CO \\ CH_2O - PO - O \cdot C_2H_4 \\ \qquad\quad | \qquad\quad (CH_3)_3 \\ \qquad OH \qquad\quad HO \end{array} \Big\} N.$$

The same facts can be put more simply by comparing an ordinary fat with lecithin. An ordinary neutral fat such as those which are found in adipose tissue or milk contains only three elements—carbon, oxygen, and hydrogen. Lecithin contains the same three elements with nitrogen and phosphorus in addition. An ordinary neutral fat on decomposition links to itself the elements of water, and then splits up (is hydrolysed) into glycerin and a fatty acid ; thus stearin yields stearic acid and glycerin ; palmitin, palmitic acid and glycerin ; and olein, oleic acid and glycerin.

Fat + water

Fatty acid Glycerin.

Lecithin yields not only a fatty acid and glycerin, but in addition to these substances it gives rise to phosphoric acid, which contains all the phosphorus of the lecithin, and choline, an alkaloid which contains all the nitrogen of the lecithin.

Lecithin + water

Glycerin Fatty acid Phosphoric acid Choline.

The fatty acid in the example we have taken of a lecithin is stearic acid ; but, as already stated, the acid obtained is not

always stearic One molecule, at least, as Thudichum pointed out, is usually oleic acid. Oleic acid is an example of an unsaturated fatty acid, and hence its affinity for oxygen. It is well known that fat blackens osmic acid (osmium tetroxide); that is, it takes oxygen from it and forms a lower oxide having a black colour. Fats which are saturated, like the glycerides of stearic and palmitic acids, do not give this reaction. The black coloration which nerve fibres give with this reagent is due to the oleic acid they contain; purified lecithin prepared from brain gives it also.

The nitrogenous derivative of lecithin, *choline*, is of considerable importance, because it can be readily detected, and the presence of choline may be usually taken as evidence that lecithin, and so nervous material generally, has undergone decomposition. Choline is an ammonium base, which has the following constitution.

$$N\begin{cases}(CH_3)_3\\CH_2-CH_2OH\\OH\end{cases}$$

Its name was originally given to it because it was first separated out from the lecithin of the bile; but its chemical name is trimethyl-oxyethyl-ammonium hydroxide. It was at one time thought to be identical with the base neurine which Liebreich separated from nervous tissues, and the two are closely related, choline readily becoming converted into neurine under the influence of certain bacterial agencies. Neurine has also been obtained by Brieger from the putrefactive decomposition of flesh. Neurine only differs from choline by two atoms of hydrogen and one of oxygen; and its structure,

$$HO.N.(CH_3)_3C_2H_3$$

justifies its chemical name, which is trimethyl-vinyl-ammonium hydroxide. These two alkaloids are also closely related to two others, namely, betaine, the very slightly toxic alkaloid of the common beet, and muscarine, the highly poisonous alkaloid of the toad-stool, *Agaricus muscarius*.

But the lecithins are not the only phosphorised fats of the

brain. An elaborate research by Thudichum * led him to the conclusion that there are three groups of phosphorised fats in the brain, which he termed kephalins, myelins, and lecithins.

The *kephalins* are very soluble in ether, and do not form definite compounds with platinum or cadmium as lecithin does.

The *lecithins* are characterised by extreme instability.

The *myelins* are less soluble in ether than the kephalins, and less soluble in alcohol than the lecithins. They are also the most stable of the phosphorised fats.

In each of these rather ill-defined groups there are several members, the empirical formulæ of which were calculated. Thus among the myelins, myelin, paramyelin, and amido-myelin were separated by their varying solubility in different reagents. It would, however, serve no useful purpose to go more fully into this matter; we know little of these substances from the chemical standpoint, and still less from the physiological.

Waldemar Koch † has recently repeated some of this work, and arrives at the following conclusions:—

The phosphorised fats are found not only in nervous tissues, but in other forms of protoplasm; for instance, he separated lecithin and kephalin from yeast cells, as well as from brain. They may therefore be fairly regarded as important for cell life.

Thudichum's myelins he found in the brain in small quantities only, and did not further investigate them. He, however, devoted a considerable share of his work to kephalin, and we may therefore briefly summarise what is known of this material.

Kephalin

Kephalin may be prepared by extracting brain substance with acetone, and subsequently with ether. To the extracts alcohol is added which precipitates the kephalin. This precipitate is collected, dissolved in ether, and purified by repeated

* *Report of Med. Officer of the Privy Council*, 1874, p. 113; and *A Treatise on the Chemical Constitution of the Brain*, 1884.

† *The Lecithans*, The Decennial Publications of Chicago University, 1902, vol. x.; *American Jour. of Phys.*, 1904, vol. xi., p. 303.

solution and reprecipitation. Thudichum ascribed to this principal member of his kephalin group, the formula $C_{42}H_{79}NPO_{13}$, and among its decomposition products identified choline, and a fatty acid he termed kephalic acid. He considered that kephalic acid took the place of the oleic acid of lecithin.

Koch's analytical figures differ somewhat, though not very greatly, from Thudichum's:—

	C	H	N	P
Thudichum . .	60	9.38	1.68	4.27 per cent.
W. Koch . .	59.5	9.7	1.78	3.84 „ „

The most important difference is in the phosphorus, which, according to Koch, is present in the same amount as in lecithin.

All we can at present say about kephalin is that it is a member of the group of phosphorised fats, and it is possible as Koch suggests that it may be a stage in lecithin metabolism. Whether kephalic acid is a new fatty acid, or merely oleic acid contaminated with other fatty acids, it is impossible to say.

Miss Tebb has recently in my laboratory prepared from brain considerable quantities of lecithin and kephalin, with a view to settling some of these vexed points if possible. Kephalin gives the osmic acid reaction in a very intense way; so if its fatty acid is not oleic, it is probably a member of the same series.

The Cerebrins

These are nitrogenous substances which are found in the white substance (especially in the medullary sheaths), and also in egg yolk, pus corpuscles, spleen cells, etc. Several members of the group have been separated and analysed since they were first described by Müller.* Müller's formula for cerebrin is $C_{34}H_{33}NO_6$. Thudichum's phrenosin ($C_{34}HNO_8$), and kerasin ($C_{46}H_{91}NO_9$) fall into the same group, as does also Gamgee's pseudo-cerebrin ($C_{44}H_{92}NO_8$). At present, however, these materials have not advanced into the area of practical medicine. Suffice it to say that the other name of cerebroside given to the group indicates that they are glucosides, and that the sugar

* *Annal. d. Chem. u. Pharm.*, vol. cv., p. 361, 2nd Abth.

(cerebrose) obtainable from them has been identified as galactose almost simultaneously in this country and in Germany.*

Gamgee † was the earliest to contest Hoppe-Seyler's view that protagon is a mere mixture which can be separated by the action of solvents into a phosphorised substance, and a non-phosphorised cerebrin. He, however, admitted that by more vigorous means (action of caustic baryta) cerebrins are obtainable from protagon; of which in particular he studied the one he named pseudo-cerebrin. Recently Woerner and Thierfelder ‡ have isolated a substance they called cerebron, by treating protagon with alcohol containing benzene. Cramer points out that this is probably pseudo-cerebrin under another name; their analytical figures certainly closely agree with those of Gamgee.

The Cerebro-Spinal Fluid

Any account of the chemical structure of the brain would be incomplete without some reference to the cerebro-spinal fluid, and I propose to conclude this lecture by a brief description of this remarkable fluid.

Most of our knowledge of the cerebro-spinal fluid has been derived from an examination of the contents of meningoceles, and in cases of hydrocephalus. The fluid removed by the first tapping at all events may be regarded as fairly normal. A few years ago, however, I had the opportunity of examining the fluid from a remarkable case which was under the care of Dr St Clair Thomson. The patient was a young woman who had for years suffered from continuous dripping from the nose; this was not amenable to any treatment. At first it was thought to be a case of nasal hydrorrhœa, but certain characters in the affection convinced the observer that this could not be so, and that the fluid, which dropped from one nostril only, was cerebro-spinal fluid. This was supported by the results of the chemical examination of the fluid.

The escape of cerebro-spinal fluid from the nose has long been known to follow traumatic injury to the cribriform plate

* Brown and Morris, *Proc. Chem. Soc.*, London, 1889, p. 167; Thierfelder *Zeit. f. physiol. Chem.*, vol. xiv., p. 209.

† *Phys. Chem.*, vol. i., p. 440 *et seq.*

‡ *Zeit. f. physiol. Chem.*, 1900, vol. xxx., p. 542.

of the ethmoid bone, but the possibility of its spontaneous escape from the nose does not appear to have been fully established before the present instance. However, considerable research into the literature of the subject has shown that there are several cases recorded in which, though no history of injury existed, the flow of fluid from the nose was of such a character that they must have been similar to the present case, although in the majority of instances the true nature of the fluid escaped observation. Many of these patients exhibited cerebral symptoms in the course of the disease, and some ultimately died from inflammation of the cerebral meninges, which had probably spread from the nose through some opening in the bony lamina that normally separates the cranial and nasal cavities.*

The first inquiries we instituted in Dr Thomson's case related to the quantity of fluid formed. One portion, collected by the patient herself in the course of an hour, measured 4 c.c. Another portion, collected under the supervision of Dr Thomson in ten minutes, measured 3.9 c.c.

If the first portion is taken as a measure of the rate of secretion, the amount formed in the day will be 96 c.c. Taking, however, the second observation as being more accurate, the amount formed in the twenty-four hours will be over half a litre (561.6 c.c.). It is possible that this estimate is too high, as doubtless the patient, being under the observation of a physician, would be somewhat excited, and the consequent alteration of the circulation would, as we shall immediately see, cause the flow to become more abundant.

In a monograph on the cerebral circulation † Hill put forward the view that the rate of secretion of the cerebro-spinal fluid, when the cranio-vertebral cavity is opened, depends directly on the difference between the pressure in the cerebral capillaries and that of the atmosphere. At the same time it was shown that

* "Observations on the Cerebro-spinal Fluid in the Human Subject," by St Clair Thomson, M.D., Leonard Hill, M.B., F.R.S., and W. D. Halliburton, M.D., F.R.S., *Proc. Roy. Soc.*, vol. lxiv., p. 343.

A full account of the case is given in Dr Thomson's book, *The Cerebro-spinal Fluid.* Cassell & Co., 1899.

† *The Physiology and Pathology of the Cerebral Circulation*, by Leonard Hill. London: Messrs Churchill, 1896.

cerebral capillary pressure varies directly and absolutely with vena cava pressure Thus the cerebral capillary pressure can be raised with great ease by any agency which causes a rise of pressure in the vena cava or cerebral veins. On the other hand, cerebral capillary pressure varies directly, but only proportionately, with aortic pressure, for between the aorta and the capillaries there lies the peripheral resistance.

It follows from the above that the easiest methods of raising the cerebral capillary pressure in man are: (*a*) By compression of the abdomen. (*b*) By the assumption of the horizontal posture. In this position, however, the rise of venous pressure may be compensated by the fall of arterial pressure, which normally occurs when the body is at rest; this is, no doubt, the case during sleep. (*c*) By straining or forced expiratory effort, with the glottis closed.

By all these methods the vena cava pressure is considerably raised; and by the last method the venous inlets into the thorax may be completely blocked, and the pressure in the cerebral capillaries raised to something like aortic pressure.

It is true that by such a forced expiratory effort the aortic pressure is lowered. Nevertheless, the total effect on capillary pressure is a very great rise, for a fall of aortic pressure of 25 mm. of mercury produces a fall in cerebral capillary pressure of less than 5 mm. of mercury, while a rise of vena cava pressure of 25 mm. of mercury produces a rise of cerebral capillary pressure of 25 mm. Hg.

Dr Thomson's case gave us a unique opportunity of testing the correctness of these views on the living human subject, and our experiments entirely confirm them. As will be seen from the following figures, the flow of cerebro-spinal fluid is accelerated by all those circumstances which raise the cerebral capillary pressure.

The fluid passed while the patient was making forced expiratory efforts was nearly double in quantity that which flowed while she was sitting quietly. Abdominal compression also raised the rate of flow, by increasing the vena cava pressure and so leading to increase of the cerebral capillary pressure. In all cases increase of volume is accompanied with fall in the percentage of solids in the fluid.

The following table illustrates these points :—

Condition of Patient.	Amount of fluid collected in ten minutes.	Percentage of solids in the fluid.
1. Sitting quietly	2.378 c.c.	1.1
2. During straining	3.912 c.c.	0.43
1. Sitting quietly	2.188 c.c.	1.14
2. Abdomen compressed . . .	3.009 c.c.	0.68
1. Sitting upright	1.670 c.c.	1.11
2. Lying down	3.245 c.c.	1.03

Cavazzani,* from experiments on dogs, found that the cerebro-spinal fluid collected in the morning was more alkaline than in the evening, and contained more solid residue. He considers that this is related to the activity of the nervous system, and that it confirms Obersteiner's theory of sleep. He obtained corresponding results in the case of a man with traumatic fistula of the frontal bone.

We considered it worth while to repeat this observation.

The qualitative examination of the fluid collected first thing on several mornings gave the same results as that of specimens collected the last thing in the evening. Both were distinctly alkaline to litmus, but no estimation of the relative alkalinity was made. The following table gives in percentages the results of the quantitative analyses :—

	Morning Fluid.	Evening Fluid.
Water	99.004	99.027
Solids	0.996	0.973
Organic solids	0.118	0.100
Inorganic solids	0.878	0.873

The evening fluid is thus slightly poorer in both classes of constituents than that of the morning; the difference is chiefly

* "Sul Liquido Cerebrospinale," *La Riforma Medica*, Anno VIII., 1892, vol. ii., p. 591.

due to an alteration in the organic solids. This is just what we should expect, because the decreased capillary pressure during sleep would lessen the rate of exudation of water. Without committing ourselves to any theory on nervous activity or sleep, we may say that our experiments confirm those of Cavazzani.

All our experiments, therefore, show the close correspondence between the amount of the fluid and the height of cerebral capillary pressure. But in spite of this, cerebro-spinal fluid is not a simple pressure exudation from the blood. The idea that it is a secretion was first propounded by Carl Schmidt many years ago, long before the birth of Heidenhain's theory that all lymph must be regarded as a secretion, in the formation of which the endothelial cells of the capillaries play a selective action.

Schmidt propounded his doctrine on the strength of his analyses of the saline constituents of the fluid; he stated that potassium salts are more abundant than those of sodium. But this has not been confirmed by subsequent investigators. Thus Yvon* gives the following numbers, NaCl 7.098, and KCl 0.033 per 1000. F. Müller† gives the relationship of NaCl to KCl as 21.5 : 1. My own figures‡ in cases of meningocele show in 100 parts of chlorides that 95.15 consist of sodium chloride, and 4.85 of potassium chloride.

The amount and proportions of the salts are thus about the same as in blood, lymph, and transudations generally.

But examination of the organic solids shows Schmidt's contention that cerebro-spinal fluid is a secretion to be correct, though the grounds on which he supported his idea are incorrect.

The fluid stands apart from all other similar fluids in the following particulars:—

(1) Its clear, watery character.

(2) Its low specific gravity (1004 to 1007).

(3) It only contains a trace of proteid; the characters of this are those of a globulin, whilst in some cases a small admixture of proteose is present. Albumin and fibrinogen are absent.

* *Jour. de Pharm. et de Chemie*, fourth series, 1877, vol. xxvi., p. 240.

† *Mittheil. a. d. Würzburger med. Klinik*, vol. i., p. 267.

‡ *Jour. of Phys.*, vol. x., p. 232.

(4) The presence in it of a substance which reduces Fehling's solution.

For some reason or other this does not readily give the fermentation test with yeast. Hence for many years the statement was current that it could not be sugar. At one time, from the examination of a case in which a large amount of fluid was at my disposal, I put forward the hypothesis that the reducing substance was an aromatic body allied to pyrocatechin. This illustrates the danger of drawing conclusions from an insufficient number of observations. Nawratski,* however, with new methods at his disposal for identifying sugars, was able to definitely prove that in the calf, the reducing substance is dextrose. Since then, other observers have had no difficulty in substantiating Nawratski's statement in regard to the fluid of other animals, man included.

The following analyses (in parts per 1000) of the fluid from spina bifida cases may be next given† :—

In parts per 1000.	Case 1.	Case 2.	Case 3.
Water	989.75	989.877	991.658
Solids	10.25	10.123	8.342
Proteids	0.842	1.602	0.199
Extractives }	9.626	0.631	3.028
Salts }		7.890	5.115

The percentage of organic solids is thus as a rule a little higher than in the absolutely normal fluid. In cases of hydrocephalus the percentage of solids is rather greater (see next table).

In parts per 1000.	Case 1.	Case 2.	Case 3.
Water	986.78	984.59	980.77
Solids	13.22	15.41	19.23
Proteids and extractives	3.74	6.49	11.35
Salts	9.48	8.92	7.88

In cases of chronic hydrocephalus, the fluid removed by the first tapping has the normal qualitative characteristics of

* *Zeit. f. physiol. Chem.*, 1897, vol. xxiii., p. 523.

† W. D. Halliburton, "Report of Spina Bifida Committee," vol. xviii. of *Clin. Soc. Transactions.*

cerebro-spinal fluid; but that removed by subsequent tappings resembles a dilute transudation from the blood, and if inflammation supervenes this becomes more marked; the proteids become more abundant, and resemble those found in blood and lymph; the amount of reducing substance increases also. This is illustrated in the following table:—

Case of Chronic Hydrocephalus.

	Specific Gravity.	Percentage of Proteids.	Reducing Substance.
First tapping . .	1006	0.045	Traces.
Second tapping . .	1010	0.069	Fairly abundant.
Third tapping . .	1010	0.272	More abundant.

It is an interesting question whether the fluid has the same composition in all parts, for the fluid has a double origin. It is found in the lymph channels and spaces of the brain and cord tissue, and the perivascular lymphatics have been shown to open into the subarachnoid space. In the second place, it is found in the cerebro-spinal cavity (ventricles of brain and central canal of cord), and it can hardly be doubted that it is here formed largely by the secretory epithelial cells which cover the choroid plexuses.* We can only surmise that this double method of formation may imply a difference in the composition of the fluid formed. The fluid as usually examined must be a mixture of the two, and I cannot see that we have at present any certain method of collecting the two fluids separately.

I will conclude with one more question, and that is, whether choline occurs in normal cerebro-spinal fluid. The importance of this question arises from the fact that choline is a substance which lends itself readily to detection, and its presence is a valuable indication of a breakdown of nervous tissue. In a fluid which plays the part of a lymph, we naturally look for the products of disintegration of any tissue. Mott and I have shown that in diseased conditions in which the katabolic side of

* See article on Meningitis, by Dr Lees and Sir T. Barlow, in Allbutt's *System of Medicine*.

nervous action is preponderant, choline is found in great abundance. This is a point I shall have to dwell upon more fully later. For the present it is sufficient to say that in the normal fluid so little is present that it may be regarded as absent for all practical purposes. Still it is present. This Gumprecht * has shown to be the case; he worked with larger quantities of fluid than were used by Mott and myself. Although the quantity in the normal fluid is so small, it is not without interest, for it furnishes evidence that in the metabolism of the nervous tissues lecithin as well as proteid is in a condition of unstable chemical equilibrium.

The osmotic relationships of cerebro-spinal fluid are different from those of ordinary lymph.

Thus Zanier † finds in the ox that the fluid is hypertonic compared to the serum of the same animal. Widal, Sicard, and Ravant ‡ arrived at the same result by the cryoscopic method. This character separates it from other serous fluids, and various drugs pass from the cerebro-spinal fluid into the blood.

M. Lewandowsky § has performed somewhat similar experiments; he regards the fluid as a specific product of the brain, and only to a small extent as a simple transudation from the blood.

The experiments of F. Ransom || with tetanus toxin and antitoxin show that these organic materials will pass from the cerebro-spinal fluid to the blood, though they pass in the opposite direction from the blood to the lymph.

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* *Verhandl. des Congr. f. innere Med.*, Wiesbaden, 1900, pp. 326-348.

† *Centrabl. f. Physiol.*, 1896, vol. x.

‡ *La Presse medicale*, October 24, 1900, p. 128.

§ *Zeit. klin. Med.*, vol. xl., p. 480.

|| *Zeit. f. physiol. Chem.*, 1900, vol. xxxi.

LECTURE VII

METABOLISM IN NERVOUS TISSUES

In my last lecture I dwelt upon the general composition of nervous structures, and in addition to giving you a long list of the chemical substances found there, with tables of quantitative analyses, devoted some time to a description of the proteids, and phosphorised constituents of nervous material.

I propose to ask you to-day to follow me in the inquiry as to the evidence we possess of metabolic activity in nervous tissues. This will involve the discussion of such questions as fatigue and sleep.

To ascertain the chemical composition of the brain when it is dead, is a task of some difficulty, but it is easy when compared with the endeavour to determine what chemical changes it undergoes while it is alive. We are only on the threshold of such chemical inquiries; still the time cannot be far distant when we shall have crossed it and opened the door to more certain knowledge. We shall find here that the experiments made for us by nature, which we call diseases, will come to our aid, for in pathological conditions we have not the nicely balanced equilibrium between anabolism and katabolism which characterises the physiological state, but as a rule the katabolic side predominates, and so we are enabled to grasp it.

Very often, for the purposes of teaching and illustration, we compare the nervous system to a telegraphic system, penetrating to every part of a country and serving for the regulation and ordering of the various occurrences which take place there. Messages fly to and from distant parts, and are received, co-ordinated or started at central offices, which we may compare to the groups of nerve-cells we call nerve-centres. In such a

telegraphic system, the most active parts are the offices; it is there we look for evidence of action in the shape of fatigue in the operators, or wear and tear of instruments. The wires are, comparatively speaking, passive transmitters; they undergo but little change, and do not manifest signs of fatigue.

So it is in the nervous system; the signs of action are to be found in the beginnings and endings of the nerve-fibres, the cells of brain and cord, and the end organs in muscle and other peripheral structures. Any evidence of fatigue in the more passive transmitters, the nerve-fibres, is very difficult to discover. This coincides with the arrangements of the vascular supply of such parts. The nerve-centres are richly supplied with blood-vessels, which furnish them with an abundant supply of nutrient material. Cerebral anæmia rapidly produces pathological changes in the nerve-cells, and death quickly supervenes. But in a nerve the blood-vessels are comparatively insignificant, and a nerve can be removed from the body, and be made to manifest activity for many hours subsequently; though, even here, as Verworn, Baeyer, and Fröhlich have shown, oxygen is essential. The question arises here, as in muscle, whether the oxygen is more important for the anabolic or the katabolic side of nervous metabolisms.

The necessity for oxygen, and the fact that it is used up during the activity of the brain, can be very strikingly demonstrated by an experiment which Hill performed with the help of methylene blue. Ehrlich was the first to show that if a solution of this pigment is injected into the blood stream of an animal, the blood is rendered blue, but the organs, especially those which like glandular organs are in a state of activity, are colourless. On exposure to oxygen after the organs are removed from the body, they also become blue. The meaning of this is, the seat of oxidation is in the tissues and not in the blood. Though methylene blue holds its oxygen more firmly than oxyhæmoglobin does, the tissues are nevertheless able to take oxygen from it and form a colourless reduction product; but after the tissues are removed from the body, and consequently are losing this vital avidity for oxygen, they become blue once more on exposure to the atmosphere.

Now, in an anæsthetised animal the brain is inactive, and the brain, like the blood, has a blue tint. If, however, a spot of the cerebral surface is stimulated, that part of the brain is thrown into action, oxygen is used up, and the methylene blue is reduced, and in consequence that area of the brain loses its blue colour. If the animal is so deeply narcotised that the brain does not discharge an impulse, the part stimulated remains blue.

In any plan of research on changes in nerve, we must be largely guided by what is already known of the tissue to which it is so closely related, namely, muscle.

When a muscle is active, the changes it undergoes are numerous and easy to detect. The naked eye can see its shortening; the microscope reveals changes in its constituent sarcous elements. The production of heat is so prominent that a temporary rise of temperature can be ascertained to occur even with such a rough instrument as a thermometer, though for the finer changes in the temperature of small muscles a thermopile is necessary. Accompanying these manifestations of a transformation of energy, the galvanometer shows us an electrical variation; and the basis of all the other changes is the sudden and massive increase of its normal chemical tone.

Turning to nervous tissues, what a contrast we have. When active, no change is visible to the highest powers of the microscope; the refractive index of the axis cylinder remains unaltered;* the most delicate thermopile fails to detect any rise of temperature, and the chemical changes which occur are proved to take place rather by circumstantial than by direct evidence. The only change in an isolated nerve which can be detected by physical means is the electrical variation.

The chemical changes that occur on the death of a muscle are in part an exaggeration of those which take place when it is active during life. This is a guide to us when we seek to determine the corresponding facts in nerve. Rolleston† showed that in nerve there is on its death a rise of temperature. Now this can only be due to increased chemical action, and probably

* Grose, Pflüger's *Archiv*, vol. xlvi., p. 56.

† *Jour. of Phys.*, vol. xi., p. 208.

of the same kind as, though greater in degree than, that which occurs during life. Moreover, nervous tissues become acid when they die.

But in order to systematise the description of these changes, it will be best to consider them under the following headings :—

(1) The reaction of nervous tissue.

(2) The hypothetical production of carbonic dioxide during the activity of nerve.

(3) Evidence of metabolic activity in nervous structures derived from the examination of cerebro-spinal fluid, and saline extracts of nervous tissues.

(4) Evidence of metabolic activity in nervous centres derived from histological examination of nerve-cells.

(5) The absence of fatigue changes in nerve-fibres.

(6) Sleep and narcosis.

Reaction of Nervous Tissues

Heidenhain * and Gschleidlen † both state that the normal reaction of the axis-cylinder is alkaline; but on death or on long-continued activity the reaction becomes acid. They further state that the grey matter is acid even during life. O. Langendorff ‡ found the reaction of the central nervous system alkaline during life; the alkalinity rapidly diminishes after death, or on stoppage of the circulation. S. Moleschott and Battistini § found both central and peripheral portions of the nervous system acid, especially the grey matter; this was increased by activity.

I am convinced that these conflicting statements are in part due to the fact that the nervous structures in question were not always examined in the perfectly fresh condition, and they may also be partly explained by the use of different indicators of acidity by the various observers.

In my own work, I have found in animals that the fresh

* *Centralbl. f. d. med. Wiss.*, 1868, p. 833.

† Pflüger's *Archiv*, vol. viii., p. 171.

‡ *Neurol. Centralbl.*, 1885, No. 14. *Centralbl. f. d. med. Wiss.*, 1886, No. 25. See also Müller and Ott, Pflüger's *Archiv*, 1904, vol. ciii., p. 493.

§ *Arch. ital. de biol.*, vol. viii., p. 90. *Chem. Centr.-Bl.*, 1887, p. 1224.

tissues are invariably alkaline, but on exposure they become rapidly acid, especially the grey matter. In the human brains I received from the post-mortem room the reaction of the grey matter was always, and of the white matter often, acid to litmus. This I attribute to changes after death, for at least twenty-four hours had always elapsed since death. The acidity is due to lactic acid; but according to Müller and Gscheidlen it is not sarcolactic acid but the fermentation lactic acid. Müller also obtained traces of formic acid.

In order to test the question of whether acidity develops on activity, Brodie and I investigated what occurs in a non-medullated nerve. This appeared to us the best means of attacking the problem, for the possibly masking effect of a large mass of myelin would then be absent. The splenic nerves of the dog, which are large and easily dissected out, were used, but we found that after faradisation for six hours the reaction never became acid to litmus.

The Hypothetical Production of Carbon Dioxide during the Activity of Nerve

This is an interesting branch of the subject, which we owe to Dr Waller. Waller uses as his object of attack isolated nerves, usually the sciatic nerves of frogs. He stimulates them in the usual way by induction shocks, and he takes their electrical response as his guide to their activity. He has in this way studied the influence of numerous reagents and drugs upon nerve, and the presence and extent, or the absence of the galvanometric answer show whether any particular reagent increases, diminishes, or annuls nervous action. Among the reagents which he thus investigated, carbonic acid is one, and his results with this gas are most instructive. Large doses of carbonic acid act like an anæsthetic, and completely abolish the electrical response, but the nerve soon recovers when the poisonous gas is replaced by air. Very small doses of carbonic acid increase its activity, and the swing of the galvanometer needle is increased when the nerve is thrown into action. A nerve thus forms a very delicate test object for this gas; far

more delicate, in fact, than most chemical reactions are. When a nerve is excited to activity, the electrical responses improve; just as when a muscle is made to undergo a succession of contractions, the beneficial effect of contraction manifests itself by what is technically called the "staircase phenomenon." This beneficial effect of previous action is exactly similar to what is produced by minute doses of carbonic acid gas, and Dr Waller argues from his experiments that they prove what cannot be directly tested by the rougher methods of chemical analysis, namely, that activity is associated with the discharge of carbon dioxide. I shall have to return to this question in our subsequent discussion of fatigue.

Evidence of Metabolic Activity in Nervous Structures derived from the Examination of Cerebro-Spinal Fluid, and of Saline Extracts of Nervous Tissues

We are so accustomed to associate the word metabolism with the activity of the proteid constituents of protoplasm, that we are sometimes apt to forget that other materials frequently exhibit a similar alternate or simultaneous series of anabolic and katabolic phases. In nervous structures this is particularly true for their complex phosphorised molecules. Even those who like Thudichum have approached the question from the purely chemical standpoint, have drawn attention to the lability of lecithin. Gumprecht has shown that perfectly normal cerebro-spinal fluid contains minimal traces of choline, a substance derived from the decomposition of lecithin, and other phosphorised fats. This trace of choline represents the small balance on the wrong side of the account. This difference becomes much more pronounced in diseased conditions. This point I am reserving for fuller study in a future lecture.

Exactly similar evidence is obtained by making saline extracts of perfectly fresh nervous tissues. Violent reagents which break up the nervous tissues will naturally lead to the appearance of large quantities of choline in the extract mixed with numerous other substances. But physiological saline solution, the most harmless of all reagents, will even at room temperature extract

choline from perfectly fresh tissues in sufficient quantities to render its detection by both chemical and physiological tests a comparatively easy task. I do not wish to dwell at this point on the methods adopted for the detection of choline. That we shall go into quite fully later. I will only say that its most characteristic physiological action when injected into the blood stream of an anæsthetised animal, is a fall of blood pressure; when, however, the animal has received a previous dose of atropine, the fall is absent, or may be replaced by a rise of pressure when choline is injected. This is illustrated by the next two figures (Figs. 4 and 5).

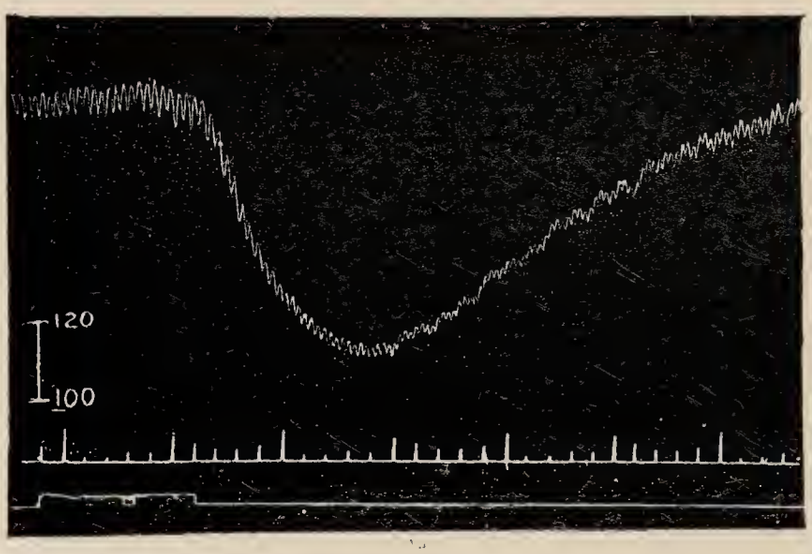

FIG. 4.

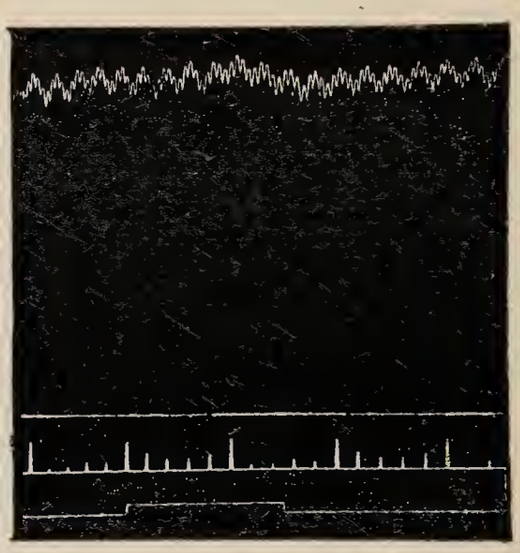

FIG 5.

Fig. 4.—Effect on injecting extract of cat's brain before atropine.

Fig. 5.—Effect after atropine of the same extract in the same animal (cat); the fall of blood pressure seen in Fig. 4 is here replaced by a slight rise. With pure solutions of choline the rise is generally more marked.

Both these figures are considerably reduced in size; the amount of reduction can be judged from the measure of the height of blood pressure indicated in millimetres on the side of Fig. 4.

This and all subsequent tracings are to be read from left to right.

The time tracing marks seconds.

The rising of the signal (lowest) line indicates the period during which the injection was being made into the external jugular vein.

The presence of choline in these extracts is a very positive sign of chemical activity in the living nerve structures; some of the phosphorised fat has undergone katabolic changes. In addition to this fact there is a further one, namely, that the most

active part of the nervous sytem, the grey matter, yields most choline to solvents.

I originally took up the subject of the physiological effects of injecting extracts of brain and other nervous tissues * in consequence of a paper by Cleghorn.† He found that extracts of sympathetic ganglia produced a fall of blood pressure, and stated that extracts of other nervous tissues did not behave in this way. As this seemed to me very remarkable, I repeated his experiments ; Osborne and Vincent ‡ did so also, and we both found that all nervous tissues behave similarly. Substances that produce a fall of blood pressure are obtained in extracts of many tissues,§ but in the majority of cases, these "depressor" substances have not been chemically identified. It is, however, probable that in some instances, the depressor substance is inorganic ; potassium and ammonium chloride both produce a fall of blood pressure, which is unaffected by atropine. The question is, Are there any other depressor substances besides choline in extracts of nervous tissues? Vincent and Cramer || have shown that there are ; the choline exists according to them in the condition of di-choline anhydride (which is a small refinement of no importance), and being soluble in absolute alcohol can be separated almost completely from the inorganic depressor materials (ammonium and potassium chlorides).¶

Evidence of Metabolic Activity of Nervous Centres derived from Histological Examination of Nerve-Cells

We know that in a muscle-nerve preparation, fatigue is due to the accumulation of the products of muscular activity, and that it may be artificially induced by irrigating such a preparation with a dilute solution of sarcolactic acid, and removed by neutralising this with salt solution containing a little alkali. It has been further shown that the muscular fibres are not to any

* *Jour. of Phys.*, vol. xxvi., p. 229.

† *American Jour. of Phys.*, vol. ii., p. 471. *Jour. of the Boston Soc. of Med. Sciences*, vol. iv., p. 289.

‡ *Jour. of Phys.*, vol xxv., p. 283.

§ Vincent and Sheen, *ibid.*, vol. xxix., p. 242.

|| *Ibid.*, vol. xxx., p. 143.

¶ Read also in this connection Gulewitsch, *Zeit. f. physiol. Chem.*, vol. xxvii., p. 50. Gumprecht, *loc. cit.* Hunt, *Proc. Amer. Phys. Soc.*, 1899. The veratrine-like action on voluntary muscles described by Cleghorn in his extracts of sympathetic ganglia is due not to choline, but to the glycerin he used as the extracting agent (Lyle, *Proc. Phys. Soc.*, 1901, p. xxvi. ; *Jour. of Phys.*, vol. xxvi.).

great extent the seat of fatigue, and that nerve-fibres are inexhaustible. By a process of exclusion, the seat of exhaustion has thus been localised in the intra-muscular nerve-endings. But when a muscle is fatigued in the intact body, there is another factor to be considered beyond the mere local poisoning of the end-plates. This is the effect of the products of muscular katabolism passing into the circulation and poisoning the central nervous system. It is stated that the introduction of the blood from a fatigued animal into the cerebral circulation of a second animal will produce in the latter all the signs of fatigue. The blood still remains alkaline; the toxic material cannot therefore be free lactic acid, and lactates do not produce the effect. Mosso has advanced the theory that the poisonous substance or substances are basic, but we have really no accurate knowledge of their nature.

There is considerable discussion just now in the scientific world on the relative importance of central and peripheral fatigue. The workers at the Brussels school maintain that the peripheral factor is the more important. Those at Turin under Mosso are prominent adherents of the doctrine of central fatigue.

Many years ago, Waller* invented an instrument which he called the *dynamograph*. It consists of a handle, which is pulled up by the hand at regular intervals against a strong spring; the amount of movement is recorded by a writing lever, upon a slowly revolving drum. Mosso some years later invented a modification of this instrument, in which the movements of a finger in raising a weight are similarly recorded. Since then several modifications of the *ergograph* (to adopt Mosso's term) have appeared.† Those who have worked with such instruments conclude that diminished voluntary power occurs at a time when the excitation of nerve or muscle gives no sign of ordinary fatigue at the periphery.

* *Brit. Med. Jour.*, 25th July 1885.

† At the actual lecture at the University of London, some of these instruments (Waller's, Mosso's, Cattell's, and Porter's) were exhibited. Dr Waller was kind enough to submit himself to an actual experiment carried out with his own instrument.

Among the recently introduced methods of examining nerve-cells, that of Golgi (the silver-chrome process), and of . issl (the methylene-blue process) stand out in special prominence. The question we have now to ask is whether these micro-chemical methods show any changes in nerve-cells as a result of activity. If they do, we have a most valuable piece of evidence in favour of the view that fatigue in the central nervous system is an important factor in the causation of what after all is a complex phenomenon, which is doubtless produced in several ways. Of the two methods mentioned, Golgi's is useless for the purpose; the methylene-blue reaction is far more delicate, and is the only one which is really helpful from this point of view.

We need hardly discuss the question whether the granules called after Nissl are present as such in healthy nerve-cells, or are produced by the alcohol used in the preliminary hardening. Healthy nerve-cells, fixed and stained in a constant manner, form the equivalent of such cells during life. It follows that if cells prepared by the same method show a difference from the equivalent or symbol of healthy cells, the difference is a measure of some change that has occurred during life.

Chromatolysis is the term applied to designate the disappearance or disintegration into fine dust-like particles of these granules. Micro-chemical methods have shown that they consist of nucleo-proteid. The name *chromoplasm* is given to this material on account of its affinity for basic dyes, like methylene blue. The name *kinetoplasm* was given to it by Marinesco in order to express the idea that it forms a source of energy to the cell. It can hardly be denied that the substance of which the granules are composed, forming as it does so large a proportion of the cell contents, and made of a material in which nuclein is an important constituent, is intimately related to the nutritional condition of the neuron.

Chromatolysis generally begins at the periphery of the cell and in the dendrons, but in advanced cases the whole cell may be affected. It occurs in various abnormal states, and under the influence of certain poisons, and its occurrence indicates a diminution of the vital interaction between the highly phosphorised nucleus and the surrounding protoplasm. Chromatolysis

alone, however, is not indicative of cell destruction, and the cell may recover its functions later when the abnormal condition passes off. The integrity of the nucleus and of the fibrils is much more important to the actual vitality of the cell.

When a nerve-fibre is cut across, the distal segment undergoes the acute change known as Wallerian degeneration. But the body of the nerve-cell and the piece of the nerve-fibre still attached to it do not remain unaffected; they undergo a slow chronic wasting, and one of the earliest signs of this *disuse-atrophy* is chromatolysis.

Chromatolysis, therefore, is a sign of inactivity; is it also a sign of excessive activity? The answer to this question is an affirmative one according to most observers.

The chromatoplasm has been compared to the granular material present in secreting cells; in such cells, before secretion occurs, the granules accumulate, and during the act of secretion they are discharged and converted into certain constituents of the secretion. In a somewhat similar way, the Nissl granules are used up during the discharge of energy from the nerve-cells, and this may be regarded as a visible sign of fatigue. The following are some of the principal observations which bear out this statement.

Eve* excited the cervical sympathetic nerve of the rabbit for twelve hours, and he found in the upper cervical ganglion that the cells presented a diffuse staining with methylene blue, which he attributes to the formation of acid substances.

A blue stain of similar appearance may be induced in the motor cells of the spinal cord after exhaustion is produced in them by giving strychnine. Max Verworn† places carbon dioxide in the first rank of the fatigue products, the accumulation of which leads to this result. It is probable that there are other fatigue products also, for after strychnine the grey matter of the cord is as a rule acid to litmus paper.

If the nerve-cells are examined after a prolonged epileptic fit in which there has been a very massive discharge of impulses, again chromatolysis is found. Some neurologists doubt whether

* *Jour. of Phys.*, vol. xx., p. 334.

† *Arch. für (Anat. u.) Phys.*, 1900, p. 152.

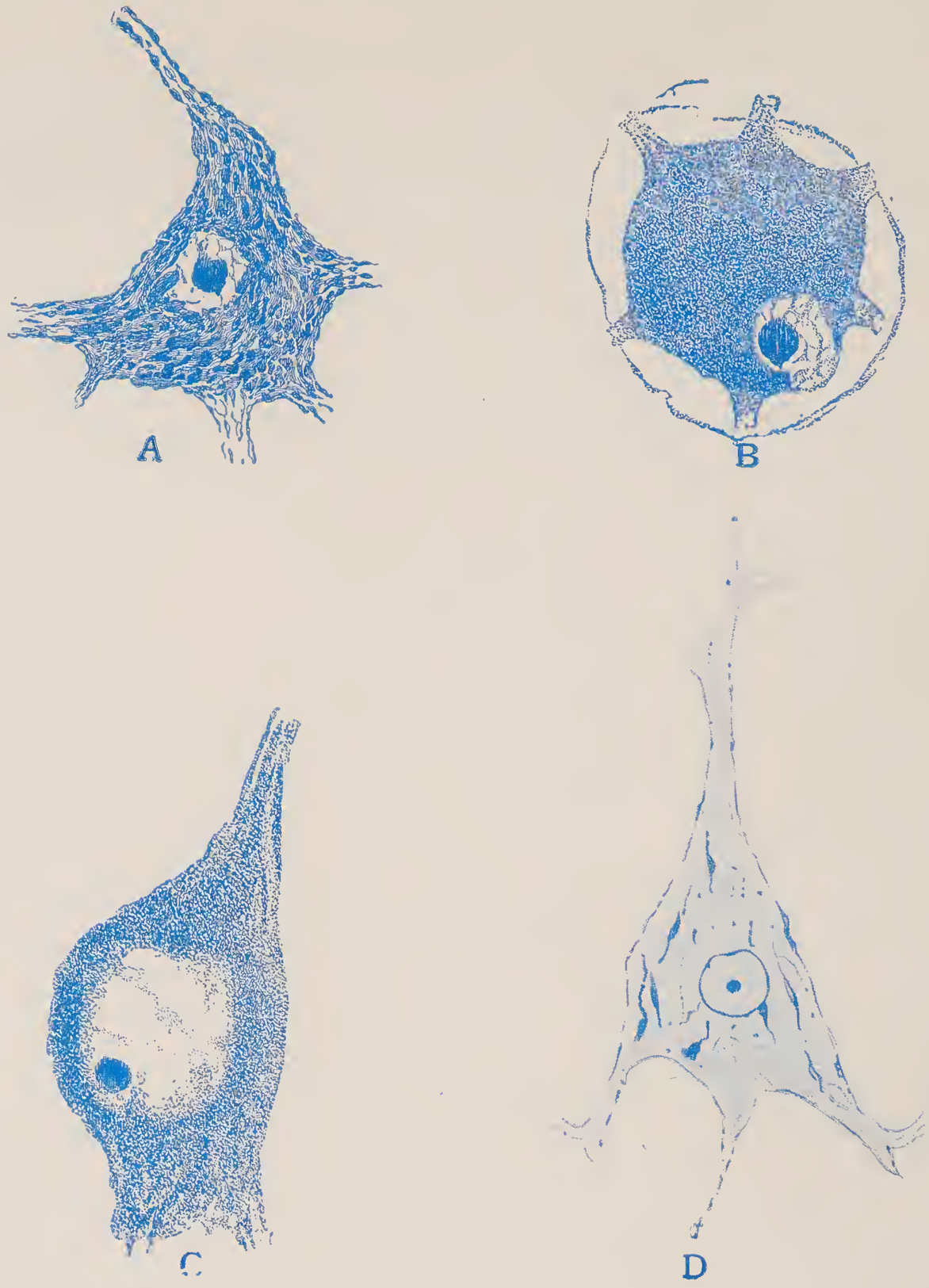

FIG. 6.—NISSL'S GRANULES.

A. Normal pyramidal cell of human cerebral cortex.

B. Swollen œdematous pyramidal cell from a case of status epilepticus. Notice diffuse staining and breaking-up of Nissl's granules into fine particles; the nucleus is enlarged and eccentric. The lymph space around the cell is enlarged.

C. Pyramidal cell of dog after ligature of vessels going to the brain, and consequent anæmia. Notice the great swelling of the nucleus and advanced chromatolysis, most marked at the periphery of the cell. Figs. A, B, and C are after Mott; magnification in each case, 700 diameters.

D. Skeleton condition of cerebral cell in a rabbit, produced by six hours' anæsthesia with ether. After HAMILTON WRIGHT.

[*To face page* 89.

this is associated with intense activity, or whether it is not wholly or in part caused by venosity of the blood. The cells are very sensitive to altered vascular conditions; anæmia, for instance, produces a similar change, accompanied by swelling of the cell, and swelling, and in extreme cases extrusion, of the nucleus.

Some very striking observations have been made on bees; the nerve-cells of animals early in the morning have been compared with those of others in the evening after a hard day's work; the very extensive chromatolysis which is noticeable in the evening animals is a very conclusive piece of evidence in favour of the view that the nerve-cells afford visible evidence of fatigue changes.

The drawings in the accompanying plate illustrate some of these appearances (Fig. 6). A is a normal cell showing the Nissl granules intact. B is a cell which shows chromatolysis as the result of the epileptic state; C shows much the same condition as a result of cerebral anæmia. D illustrates the effect of prolonged narcosis, which we shall refer to later in the course of the present lecture.

Fatigue is, therefore, demonstrable in the nerve-centres, and in the peripheral endings of nerve-fibres. Which is the more important of the two, I do not pretend to be able to decide absolutely on the evidence at present available; but having examined the evidence, I am inclined to take the view that central fatigue is the more important, and is more readily produced.

The Absence of Fatigue Changes in Nerve-Fibres

Not the least interesting of the facts we have mentioned is the non-fatigability of nerve-fibres. The experiments on which the assertion rests have nearly all been performed with medullated motor fibres. The method adopted in such experiments has been to excite the nerves for a number of hours, and to exclude fatigue in the terminal structures by preventing the impulses reaching the peripheral organ. On removing the block by means of which this is accomplished, the activity of

the peripheral organ is on stimulation of its nerve still manifested with undiminished force. The blocks employed have been curare,* a galvanic current,† the application of ether,‡ and in the case of secretory fibres, atropine.§

A few investigators have employed non-medullated fibres in their experiments. In his experiments on the cervical sympathetic, Eve‖ found that the vaso-constrictor apparatus in the ear vessels was still in action at the end of twelve hours' excitation. Here no block was used, for in the vaso-motor nerves fatigue of even the peripheral endings does not occur in demonstrable amount.

Howell, Budgett, and Leonard¶ state that vaso-constrictor and cardio-inhibitory fibres show no functional fatigue; but as the longest time during which they applied continuous excitation was only one hour, this contention can hardly be considered to be satisfactorily proved.

What does this mean? I take it that it does not mean that the nerve-fibres undergo absolutely no metabolic changes when transmitting a nerve impulse. It means that the change is so slight, and the possibilities of repair so great, that fatigue in the usual acceptation of the term cannot be demonstrated. This is an illustration of the wonderfully economic way in which Nature often works.

That a change does occur in a nerve-fibre is evidenced by the electrical variation it undergoes and which can be detected by the galvanometer or the electrometer. Further, we have already seen, Waller believes he has shown that carbonic acid is evolved by the axis-cylinder. How, then, can we account for the fact that fatigue cannot be shown to occur? To meet this difficulty Waller tentatively suggested a most ingenious explanation, which it will be well to give in his own words.** He says: "I

* Bowditch, *Jour. of Phys.*, 1885, vol. vi., p. 133.

† Berustein, Pflüger's *Archiv*, 1877, p. 289; Wedenski, *Centralbl. f. d. med. Wissensch.*, 1884, vol. xxii., p. 65.

‡ Maschek, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1887, vol. xcv., Abth. 3, p. 109.

§ Lambert, *Compt. rend. Soc. Biol.*, tenth series, 1894, vol. i., p. 511.

‖ *Loc. cit.* ¶ *Jour. of Phys.*, 1894, vol. xvi., p. 298.

** *Lectures on Physiology*, first series, *Animal Electricity*, 1897, p. 70.

wonder does this carbonic acid become altogether dissipated; may it not perhaps be reinvolved in some storage combination, as the nerve-fat, perhaps, that is so prominent a constituent of fully evolved nerve. Such nerve consists of proteid axis and fatty sheath; the axis—which is the offshoot of a nerve-cell—is the specially conductile part, the sheath is a developmental appendix, not directly connected with any nerve-cell. Yet, cut the nerve, and sheath as well as axis undergo Wallerian degeneration, which is evident proof of a functional commerce between sheath and axis. All these things to my mind reconcile themselves with the notion that the active grey axis both lays down and uses up its own fatty sheath, and that it is inexhaustible not because there is little or no expenditure, but because there is an ample re-supply."

A year or two after these words were written, Miss Sowton,* at Dr Waller's suggestion, undertook a piece of work in order to test the truth of this hypothesis. If the absence of fatigue is due to the presence of the fatty sheath, fatigue ought to be demonstrable in nerve-fibres in which the fatty sheath is absent. She selected the olfactory nerve of the pike as the non-medullated nerve with which to try the experiment, and her results confirmed Waller's expectation; the galvanometric replies of the nerve become somewhat feebler after repeated stimulation.

It appeared to me advisable to test the question in another way. Some doubt has recently been cast on the trustworthiness of the electrical response as a sign of nervous activity.† As the doubt has arisen, the greater becomes the necessity for a fresh method of attacking the problem. The splenic nerves appeared to be the most convenient large bundles of non-medullated fibres for the purpose. Dr T. G. Brodie was associated with me in carrying out the investigation. A dog is anæsthetised with morphine and A.C.E. mixture, the abdomen opened, the spleen exposed, and the splenic nerves which lie by the side of the main splenic artery are laid bare. It is quite

* *Proc. Roy. Soc.*, 1900, vol. lxvi., p. 379.

† See Professor Gotch's article "Nerve" in Schäfer's *Text-book of Physiology*.

easy to dissect out a length of nerve sufficient for the experiment (1½ to 2 inches). The nerve is then cut as far from the spleen as possible, and the spleen is enclosed in an oncometer, similar to that employed by Schäfer in his work on the spleen.* On stimulating the nerve with a weak faradic current the organ contracts, and the recording lever falls. The diminution of the size of the spleen is quite visible to the naked eye, however, without the use of any apparatus. The next thing to do is to put a block on the course of the nerve, which will prevent the nerve impulses from reaching the spleen. Here we met with some difficulty. Curare and atropine are both ineffective; the constant current has a great disadvantage; non-medullated

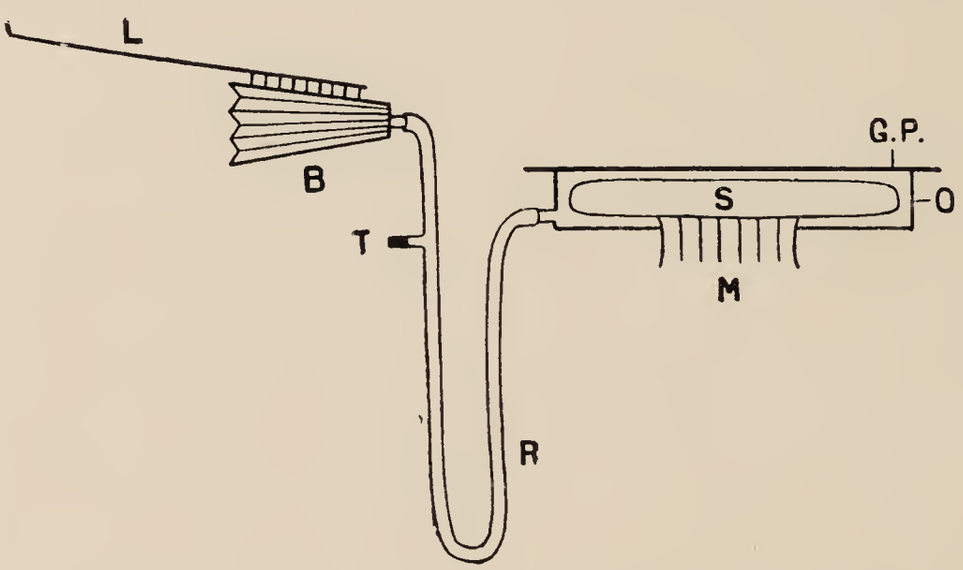

FIG. 7.—Apparatus for obtaining splenic curves. S, spleen in oncometer O, which is made of guttapercha, and covered with a glass plate (G.P.) luted on with vaseline. M is the splenic mesentery, containing vessels and nerves; this passes through a slit in the base of the oncometer, and is made air-tight with vaseline. The oncometer is connected to one of Brodie's bellows recorders (B) by the india-rubber tube (R), the side tube (T) being closed during an experiment by a piece of glass rod. The recording lever (L) writes on a revolving drum.

nerves are so much affected that very feeble constant currents (one-third of a Daniell cell) will completely block the transmission of impulses, and not only that, but the nerve remains blocked after the current is removed. After the current has been allowed to flow for two minutes the nerve remains impassable to nerve impulses for an hour or more, and then slowly

* Schäfer and Moore, *Jour. of Phys.*, vol. xx., p. 1.

recovers. If, therefore, faradic excitation of the nerve is kept up all this time and fails to excite the contraction of the spleen after the removal of the constant current, it is impossible to say whether this is due to fatigue of the nerve-fibres on the proximal side of the block, or whether it may not be due to the fact that the block created by the constant current is still effective.

Our best results were obtained by using cold instead of a constant current as our blocking agent.

Fig. 7 is an outline drawing of the apparatus used.

Fig. 8 shows the arrangement adopted in connection with the nerve. The nerve (N) rests on a metal tube (T) through which fluid can be kept flowing. E is the situation of the electrodes. If the nerve is excited, the spleen contracts and the recording lever (in Fig. 7) falls. If now brine at 0° to 2° C. is kept flowing through T, the nerve impulses are blocked by the cold, and cannot reach the spleen. Immediately the cold brine is replaced by warm water at 30° C., the nerve again becomes passable by nerve impulses, and the spleen contracts once more.

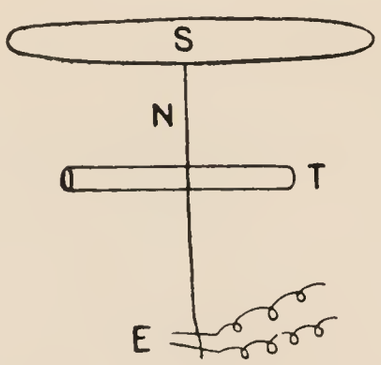

FIG. 8.—Arrangement of apparatus in connection with the splenic nerve. S is the spleen, and N the main bundle of nerves. The nerve rests on the metal tube (T), through which water at the required temperature is kept flowing, and on the electrodes (E), which come from the secondary coil of an inductorium.

If now the water in T is kept at the low temperature mentioned, and the nerve is being excited with strong induction shocks all the time, the spleen remains irresponsive; the nerve-impulses are able to reach T but not to pass it. If then warm water is passed through T, and the block produced by the cold is thus removed, and the spleen continues to be irresponsive, we have a proof that the piece of nerve between E and T has been fatigued. But our experiments have shown us that non-medullated nerve is just as difficult to fatigue as medullated nerve. Even after six hours' continuous excitation the nerve is just as excitable as it was at the start, and

a full splenic contraction is obtained when the cold block is removed.

At the lecture an experiment of this kind was shown to those present; and it may not be uninteresting to reproduce the tracings which were actually taken (at the University of London). At the commencement of the lecture, tracing B (Fig. 9), was obtained by stimulating one of the large splenic nerves. The downward movement of the lever began very soon after the faradic excitation started (the duration of which is shown by the raising of the signal line); recovery began a little later, and finally the splenic lever wrote at its former level. The cold block was then introduced, and faradic excitation was maintained until the end of the lecture, the spleen being irresponsive all the time. At the end of the lecture, about an hour and a half later, the cold brine was replaced by warm water; during this manipulation the excitation was stopped for a few moments; then excitation produced the contraction seen in tracing C, which is equal in extent to that in B. It did not last quite so long, simply because the excitation was a shorter one; the excitation was of the same strength (Daniell cell attached to primary coil, secondary coil of the du Bois-Reymond inductorium pushed right home) in both cases. The bellows recorder possesses the advantage of being capable of calibration; the contraction of the spleen in this case squeezed into the circulation about 20 c.c. of blood; this caused a slight temporary increase of arterial pressure, a tracing of which was being simultaneously recorded by a mercurial manometer attached to the carotid artery. It is a wise precaution to take such a tracing, because in prolonged experiments of this kind, the general condition of the animal can be gauged by the height of arterial pressure. I have not, however, thought it worth while to reproduce this part of the record.

A possible objection to this experiment might be made in this way. It is well known that heart muscle obeys the rule which Waller has so tersely termed "all or nothing." That is, a stimulus strong enough to provoke a contraction of cardiac muscle always calls forth a maximum contraction. If the same rule applies to the smooth muscle of the spleen, the excitation shown in tracing C might really be quite a weak excitation owing to nerve fatigue, but still sufficient to evoke a full splenic reply. Happily, this objection does not hold; plain muscle does not obey the "all or nothing" rule; within fairly wide limits the amount of contraction is roughly proportional to the strength of the stimulus. We have proved this over and over again with the spleen. During the rehearsal of this very experiment, Dr Brodie and I tried various positions of the secondary coil, and in tracing A is shown one of the responses to a weak excitation. We finally decided to push the secondary coil right home for the lecture experiment, in order to make the result as striking as possible.

In addition to our work on the spleen, we made corresponding observations on the vaso-motor nerves contained in the

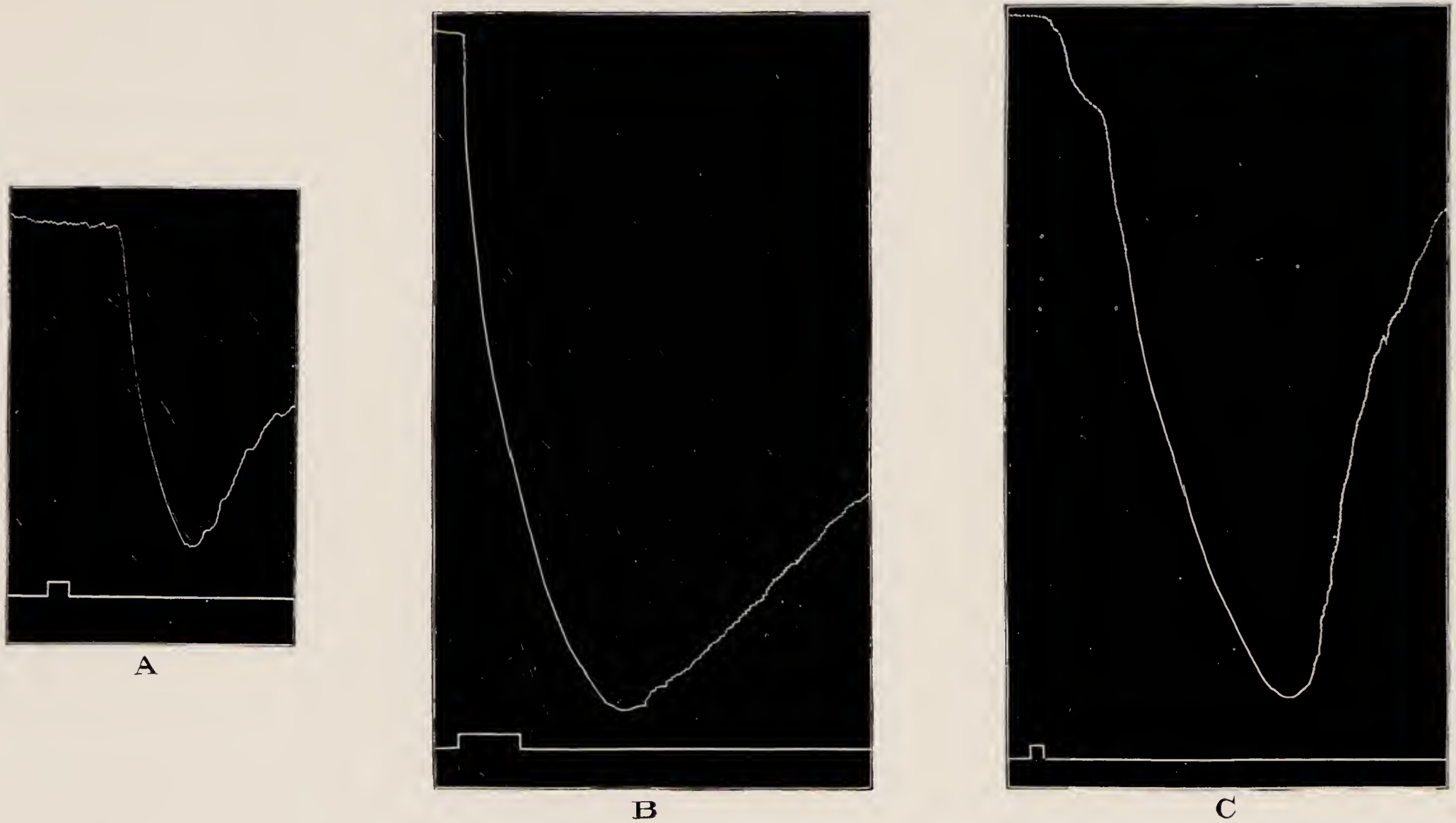

FIG. 9.—Splenic contractions indicated by the downstrokes. For description, see text.

[*To face page* 94.

sciatic nerve of the dog, the volume of the leg being recorded with a plethysmograph, and we also repeated Eve's experiments on the cervical sympathetic running to the ear of the rabbit.

In no case were we able to demonstrate any functional fatigue. But we did notice, especially in vaso-motor nerves, a phenomenon which Howell terms *stimulation fatigue;* this means that the actual spot of nerve stimulated becomes after a time less excitable, and finally, inexcitable, though it will still transmit impulses, if the excitation is applied above the spot originally stimulated. We think that the use of the term "fatigue" in this connection is a mistake; the prolonged electrical excitation causes injurious polarisation (due to electrolytic changes) of the nerve, which renders it less excitable. This view has been confirmed by Professor Gotch by means of experiments with the capillary electrometer. This so-called "stimulation fatigue" was not excluded in Miss Sowton's experiments, and will possibly explain her results. The splenic nerves, curiously enough, do not exhibit this phenomenon in any marked degree, and so were peculiarly well adapted to test the question of functional fatigue. On *à priori* grounds we should hardly expect non-medullated nerves to be peculiarly susceptible of real fatigue, when one considers how many of them, like the vaso-constrictors, are in constant action throughout life.

Our final conclusion therefore is, that although fatigue effects are demonstrable where nerve-fibres start in the nerve-cells, and also where they terminate in peripheral structures, at present fatigue has not been demonstrated to occur in the course of the fibres between these two extremities. Further, that although the possibility still remains that this is to be explained on Waller's hypothesis of "functional commerce" between the axis-cylinder and its sheath, it is not the medullary sheath which is essential, for fatigue is just as difficult to demonstrate in non-medullated nerves as in those of the medullated variety.

The experiment demonstrated at the University of London led Dr Waller to suggest to Dr Alcock that he should investigate the behaviour of the splenic nerve towards the galvanometer, and he has since then published his results;* his chief conclusions are :—

* *Proc. Roy. Soc.*, 1904, vol. lxxiii., p. 166.

1. Non-medullated nerves exhibit a negative variation and current of injury three times greater in magnitude than the medullated nerves of the same animal.

2. The negative variation (or current of action) of non-medullated nerves undergoes a progressive diminution with repeated stimuli. (Confirmatory of Miss Sowton.)

3. The immediate cause of this diminution is a localised change at the point of excitation. (Confirmatory of our suggested explanation of Miss Sowton's results.)

4. The electrotonic currents of non-medullated nerves are very small, only about one-fortieth of those in medullated nerves. (This explains 2 and 3, because the exciting current being confined to the place of application, has a greater density, and therefore a greater local effect.)

In these days when physiologists are so prone to differences of opinion, it is satisfactory that two such diverse methods as those adopted by Alcock and by Brodie and myself, have led to the same conclusion concerning the absence of true functional fatigue in non-medullated nerve-fibres.

Sleep and Narcosis

Having now studied fatigue in many of its aspects, it is appropriate that we should in conclusion turn our attention to a brief consideration of sleep, Nature's great restorer for exhausted "nerves."

Theories to account for sleep are numerous, and none are satisfactory. Thus by some it has been attributed to changes in the blood-supply of the brain, and ultimately referred to fatigue of the vaso-motor centres. The existence of an effective vaso-motor mechanism in the cerebral blood-vessels themselves is problematical; so that if changes occur in the cerebral blood-pressure or rate of flow, they are mainly secondary to those which are produced in other parts of the body. It is, however, quite possible that the vascular condition is rather the concomitant or consequence of sleep than its cause.

Some of the theories to account for sleep have been chemical. Thus certain observers have considered that sleep is the result of the action of chemical materials produced during waking hours, which have a soporific effect on the brain; according to this theory, awakening from sleep is due to the action of certain other materials produced during rest, which have the opposite effect. Obersteiner has gone so far as to consider

that the soporific substances are reducing in nature, and others regard them as alkaloidal. These theories all rest upon the slimsiest foundations, and none has yet been found to stand experimental tests.

Then there are what we may term histological theories of sleep, and these are equally unsatisfactory. The introduction of the Golgi method opened a fresh field for investigators, and several have sought to find by this method a condition of the neurons produced by narcotics like opium and chloroform, which is different from that which obtains in the waking state.

Demoor* and others found that in animals in which deep anæsthesia has occurred, that the dendrites exhibit moniliform swellings, that is, a series of minute thickenings or varicosities. On the strength of this observation, he has formulated what we may call a bio-physical theory of sleep. In the waking state, the neighbouring nerve-units are in contact with each other; transmission of nerve-impulses from neuron to neuron is then possible, and the result is consciousness; during sleep the dendrites are retracted in an amœboid manner; the neurons are therefore separated, and the result is unconsciousness.

Lugaro, on the other hand, takes the precisely contrary view. He was not able to discover moniliform enlargements, and his bio-physical hypothesis is that the interlacing of dendrites is much more intimate during sleep than during consciousness. He therefore explains sleep by supposing that the definite and limited relationships between neurons no longer exist, but are lost and rendered ineffective by the universality of the connecting paths. It is not very difficult to explain such divergence of views, for they both depend mainly on observations made by a single method; and the method itself is open to objection. It is one which gives even in the same brain most inconstant results, and is not calculated to show much more than a mere outline of a few of the cells and their branches. So much doubt has arisen of late in regard to the trustworthiness of the method, that many neurologists are beginning to doubt whether the neuron theory implying

* *Arch. de Biol.*, 1896, vol. xiv.

absolute non-continuity of nerve-units has been satisfactorily proved, and there is a tendency to return to the idea of a connecting network not very different from that originally put forward by Gerlach.

A more satisfactory investigation of the effect of anæsthetics on nerve-cells has been carried out by Dr Hamilton Wright,* who performed the majority of his experiments in my laboratory.

He used rabbits and dogs, and subjected them to ether and chloroform narcosis for periods varying from half an

A B

FIG. 10.—Moniliform enlargements on dendrites of nerve-cells, rendered evident by Cox's method. A is a cortical cell of a rabbit; B is a corresponding cell of a dog's brain, after six hours' anæsthetisation with ether in each case. HAMILTON WRIGHT.

hour to nine hours. In both animals he found that the nerve-cells are affected, but in rabbits much more readily. This accords quite well with what is known regarding the susceptibility of rabbits as compared to dogs towards the influence of these narcotising agents. In a rabbit the nerve-cells, especially of the cerebrum, show changes even after only half an hour's anæsthesia, but in dogs at least four hours'

* *Jour. of Phys.*, 1900, vol. xxvi., p. 30; 1901, vol. xxvi., p. 362.

anæsthesia must be employed. By the Golgi method (Cox's modification) the moniliform enlargements can be seen. These become more numerous, larger, and encroach more and more on the dendritic stems, the longer the anæsthesia is kept up. The accompanying illustrations (Fig. 10) show the appearances seen.

Lugaro's failure to find these appearances is doubtless due to his not having maintained the anæsthesia long enough in his dogs.

Wright started his work with a bias in favour of Demoor's bio-physical theory, but he soon found that the theory was untenable; the results of his observations have shown him that the action of anæsthetics is bio-chemical rather than bio-physical, and he has been led to this conclusion by the employment of other histological methods, particularly the most sensitive one we possess, namely, the methylene-blue reaction.

Owing to the chemical action of the anæsthetic in the cells, the Nissl bodies have no longer an affinity for methylene blue, and the cells consequently present what Wright calls a rarefied appearance; when this becomes marked the cells appear like the skeletons of healthy cells (see Fig. 6, D). In extreme cases the cells look as though they had undergone a degenerative change, and after eight or nine hours' anæsthesia in dogs, even the nucleus and nucleolus lose their affinity for basic dyes. The change, however, is not a real degeneration, and passes off when the drug disappears from the circulation. Even after nine hours' anæsthesia the cells return rapidly to their normal condition, stain normally, moniliform enlargements have disappeared, and no nerve-fibres show a trace of Wallerian degeneration. The pseudo-degenerative change produced by the chemical action of the anæsthetic no doubt interferes with the normal metabolic activity of the cell-body, and this produces effects on the cell branches. In the early stages of Wallerian degeneration, the branch of the nerve-cell which we call the axis-cylinder presents swellings or varicosities, produced by hydration or some similar chemical change. The moniliform enlargements seen during the temporary pseudo-degenerative

effects produced by anæsthetics are comparable to this.* These enlargements are therefore not the primary cause of loss of consciousness, but are merely secondary results of changes in the cell-body. When a tree begins to wither, the earliest apparent change is noticed in the branches most remote from the centre of nutrition, the root; as the changes in the centre of nutrition become more profound, the larger branches become implicated, but the seat of the mischief is not primarily in the branches. This illustration may serve to render intelligible what is found in nerve-cells and their branches.

Whether the appearances found in dogs and rabbits are applicable to the human subject, is another question. I am inclined to think that we may safely regard them as such; there is no reason why an anæsthetic should act differently in different animals. The resistance of the animal is a variable factor, and this causes a variation in degree only; the effect is probably the same in kind for all animals, man included.

But I feel that we should be very chary in concluding that the artificial sleep of a deeply-narcotised animal is any criterion of what occurs during normal sleep. The sleep of anæsthesia is a pathological condition due to the action of a poison. The drug reduces the chemico-vital activities of the cells, and is, in a sense, dependent on an increasing condition of exhaustion, which may culminate in death. Normal sleep, on the other hand, is not produced by a poison, or at any rate we have no evidence of any poison; it is the normal manifestation of one stage in the rhythmical activity of nerve-cells, and though it may be preceded by fatigue or exhaustion, it is accompanied by repair, the constructive side of metabolic activity.

Since the foregoing lecture was delivered, a very valuable contribution to the already extensive literature on the anæsthetic question has been published by B. Moore and H. E. Roaf.† They point out that although anæsthetics are very numerous, there is probably only one type of interaction

* Some observers look upon the varicosities as artifacts. If they are, they ought to have been found in all Wright's specimens, for the method of preparation was the same throughout.

† *Proc. Roy. Soc.*, 1904, vol. lxxiii., p. 382.

between the anæsthetic and cell-protoplasm. The cells usually investigated, as in Hamilton Wright's work, are the nerve-cells, naturally because the state of quiescence produced in those cells underlies the state of unconsciousness; but all other cells are similarly affected, although in varying degree. The metabolic processes in ciliated epithelium, amœbæ, bacteria, etc., are stilled as effectually as those in nerve-cells are. Hence the action of the anæsthetic must be due to a change in some substance which is uniformly present in all kinds of cell-protoplasm.

From this wide point of view, anæmia or hyperæmia of the brain described by different observers must accordingly be set down as secondary effects, and not primary causes, of anæsthesia. Similarly, theories based on the high content in lecithin, cholesterin, and fatty derivatives soluble in ether or chloroform, of the nerve-cell, cannot furnish any explanation of anæsthesia. The changes noted by Wright take time to develop, and form a signal of the changes produced in the protoplasm in a marked degree by the prolonged action of the anæsthetic.

The most constant constituent of cell-protoplasm is proteid, and accordingly the authors turned their attention in the experiments they have hitherto recorded, to the action of chloroform on proteid material. They find that unstable compounds of proteid and chloroform are obtainable. In this way chloroform is combined with the blood-proteids, and accounts for the fact that chloroform is so much more soluble in blood than in water or salt solution. The chloroform-proteid compound is compared to oxyhæmoglobin, and undergoes dissociation in the same kind of way; just as oxyhæmoglobin parts with its oxygen to the cells of the tissues, so the chloroform parts company from the blood-proteid, and enters into combination with the proteid matter of cell-protoplasm; this limits the activity of the cell-protoplasm, and produces anæsthesia. In time, when the administration of the anæsthetic has ceased, and the chloroform tension in the blood is no longer maintained, the combination between cell-proteid and chloroform dissociates, and anæsthesia passes off.

LECTURE VIII

THE COAGULATION TEMPERATURE OF THE NERVE-PROTEIDS, AND ITS BEARING ON THE QUESTIONS OF: (1) THE GALVANOMETRIC RESPONSE OF NERVE UNDER VARYING TEMPERATURES; (2) HEAT CONTRACTION IN NERVE; AND (3) HYPERPYREXIA

I HAVE already told you something about the proteids of nervous tissues (pp. 61-63), but my affection for questions of proteid chemistry has prompted me to inflict upon you a whole lecture to be devoted to certain further results of our study of these substances. These come under three heads:—

(1) The influence of temperature on the galvanometric response of nerve to stimulation.

(2) Heat contraction in nerve.

(3) The coagulation temperature of cell-globulin and its bearing on hyperpyrexia.

The Influence of Temperature on the Galvanometric Response of Nerve to Stimulation

You all know that when a nerve is stimulated to activity, it undergoes a change of electrical potential, which can be detected with the galvanometer or electrometer. This we still call by du Bois-Reymond's old name, the negative variation, although we now often express it by the newer phrase, current of action. The change briefly is that any excited spot becomes momentarily electro-positive to other parts of the nerve, and this is propagated along the nerve as an accompaniment of the nervous impulse.

herefore, we place two non-polarisable electrodes, *p* and *d*

(Fig. 11), a few millimeters apart upon a nerve, one end, A, of which we excite, and connect the electrodes to a sensitive galvanometer, immediately the impulse reaches the situation of the first electrode *p*, this point becomes positive to *d*, and, therefore, a current flows from *d* to *p* through the galvanometer. A moment later the two points are equipotential, and no current flows; a minute fraction of a second later this balance is upset, that is, when the impulse reaches the point *d*; *d* then becomes positive to *p*, and so the galvanometer needle moves in the opposite direction. If we use the electrometer, the mercury in capillary tube moves first in one direction then in the other. Hence the expression *diphasic variation.* If instead of single stimuli we employ a series of shocks as during faradisation, the galvanometric reply is more complex, but more distinctly seen, being the resultant effect of the individual stimuli.

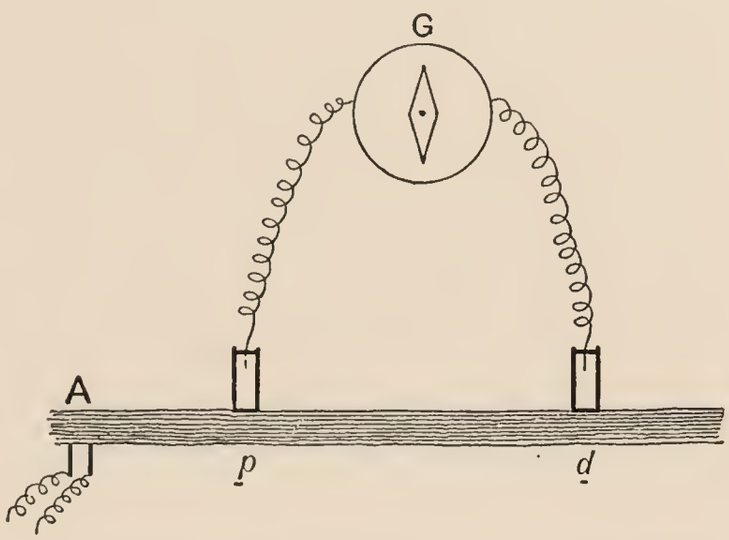

FIG. 11.

It is a remarkable fact in the history of electro-physiology, how our knowledge of the electrical changes in nerves has been constructed almost altogether from experiments on the nerves of one animal, namely, the frog.

Dr Alcock,* however, has filled in the gap, and shown that there is no essential difference between the nerves of frogs, mammals, and birds as regards their negative variation, excitability, and their susceptibility to the narcotic effects of anæsthetics.

In one particular, however, there is a difference, and one which is to myself most interesting; that is the temperature necessary to extinguish this sign of life. The nerves are paralysed by cold, and this temperature also varies, being

* *Proc. Roy. Soc.*, 1903, vol. lxxi., p. 264.

-3.5° C. in the frog, -1.4° in the hedgehog, $+3.8^{\circ}$ in the rabbit, and $+6.9^{\circ}$ in the pigeon. The influence of cold is not, however, fatal; on being warmed up again the nerves recover. The differences in these animals in the cold necessary to inhibit nervous action is interesting to the comparative physiologist in relation to the normal conditions under which such animals live. The cold-blooded frog requires much more intense cold for the purpose than the warm-blooded rabbit; and the hot-blooded pigeon is still more readily affected than the rabbit. The hedgehog is an animal which hibernates, and becomes to all intents and purposes a cold-blooded animal during its winter sleep. We see it occupies an intermediate position in the figures given, between the frog and the rabbit.

Equally interesting, or perhaps even more so, is the influence of high temperatures; the negative variation is abolished at 40° in frog's nerve, at 48°-49° in rabbit's nerve,* and at 53° C. in pigeon's nerve. I regard this fact as more interesting because we are able to explain it. The extinction point corresponds with the first coagulation point of the body proteids, and thus heat coagulation is the cause of the *permanent* loss of irritability of the nerve. You will remember, we have seen the same in muscle; loss of irritability is produced when the lowest coagulating proteid is coagulated, and there also the temperature varies in the different parts of the animal kingdom, affording an instance of what we then called biological adaptation.

Dr Alcock is happily here, and he has been good enough to get ready a typical experiment. I expect you have all seen a galvanometer experiment with frog's nerve, so he has selected a rabbit's nerve for his demonstration. We will have the lights lowered, and now you see the spot of light reflected on to the screen from the galvanometer mirror. We will first adjust it to the zero point, and throw into the circuit a thousandth part of a volt; you see the spot of light moves along the screen till it reaches division number 25, and then slowly returns to zero.

* Eve, in work on the cervical ganglion (*Jour. of Phys.*, vol. xxvi., p. 119), places the death temperature of the rabbit's nerve-cells at the same point, 46° to 49° C., and Howell made corresponding observations on the temperature necessary to abolish the conductivity of nerve in frogs and mammals.

Now, I will ask Dr Alcock to place the nerve on the non-polarisable electrodes, and to excite it with the faradic current for a few seconds, and to repeat this, always exciting for the same length of time, first while the nerve is at 30°, secondly, when he has raised the temperature to 41°, and finally, when the temperature is 49° C., and to write his results upon the blackboard.

The following was the final appearance of the table so constructed :—

Temperature of the Nerve.	Movement of spot of light in divisions of scale.
1. 30° C.	9.5
2. 30° C.	9.5
1. 41° C.	10.0
2. 41° C.	10.6
1. 49° C.	0
2. 49° C.	0
1/1000 of a volt.	25

You see quite easily how a temperature of 41° C., which is sufficient to abolish the response in frog's nerve, has a little increased the vigour of a mammalian nerve; whereas a temperature of 49° C. has killed it.

Heat Contraction in Nerve

It is possible to attack the same problem in another way. While I was lecturing to you on heat rigor in muscle, and Dr Brodie was showing us an experiment with the frog's sartorius to illustrate his method, one of those inspirations which so seldom happen to a speaker in the course of a lecture, chanced to occur to me. It was this, why should we not try the same experiment with nerve? I put the case to Dr Brodie, and we have subjected the question to the test of experiment. I am now able to tell you our results, and show you a typical experiment.* At first we were disappointed with our results; the shortening in a nerve was very small and often absent; we

* At the University of London, our work had not advanced far enough for us to actually show this experiment; I was, however, able to demonstrate it successfully at New York, where, in consequence, I decided to omit the galvanometer experiment of Dr Alcock.

however, obtained better results with the spinal cord. We soon found that the reason of failure was that we employed the same apparatus as Brodie used for muscle. This has too much resistance for a nerve to move. It is essential that the parts moved should be extremely light, and free from friction. This we have accomplished by the very simple piece of apparatus which I have on the table. I have here a trough containing mercury covered with physiological saline solution; a frog's sciatic nerve lies horizontally in the salt solution on the surface of the mercury; it is fixed at one end; the free end is attached to a light aluminium wire suspended vertically; at the upper end of this is a little mirror which reflects a spot of light on to this screen. The trough is placed in a water-bath, and carefully warmed; and as the nerve shortens, the spot of light moves down the screen.

The temperature is now 36° C., and the light has shifted already a little, but it is not until we reach 40° that the movement becomes energetic, and the spot moves over several divisions of the scale. This is exactly the same as in muscle; the small preliminary movement corresponds to what in a proteid solution we should term the stage of opalescence. We ought properly to keep the temperature at 40°-41° C. for at about half an hour, in order to ensure the thorough coagulation of all of this first proteid at that temperature. The actual amount of this proteid is small, as judged by the amount of shortening. But I cannot expect you to sit patiently here and watch a spot of light for half an hour, as Brodie and I do in our dark room. So we will continue the heating, and now at 47° the spot once more starts moving, and at 48°-49° has walked half-way down the screen. Again, you must imagine a half-hour at this temperature to elapse, and a third movement is then visible at 56° to 58° C.

We have taken the rabbit and cat as instances of mammals; the proteid in nerve coagulating at 40° C. is absent, and the first proteid produces the first shortening at 47°, and a second at 56°. There is also a third and very pronounced one at 62°-63°, which is doubtless due to the connective-tissue sheath (see coagulating point of tendon, p. 54).*

* The large amount of connective tissue in mammalian and birds' nerves limits useful observation beyond that temperature. But in the frog's nerves,

Finally, we have taken the pigeon as our instance of a hot-blooded animal (normal temperature, 42° C.), and here we find the temperature of the first step in the contraction is 50°-53° C. There is a second at 58°-59°, and a third (connective tissue) at 63° C.

Heat contraction of nerve or of spinal cord, where the same facts are true, thus occurs like heat rigor in muscle in successive steps, and these coincide with the coagulation temperatures of the proteids contained in saline extracts of nervous tissues. The ultimate length of the nerve when heat contraction is finished is usually (in the frog) about half its original length.

The method is capable of application to other tissues; we have already made some experiments with strips of rabbits' liver, and the shortenings at 47° and 56° correspond with the coagulation points of the two principal proteids found in saline extracts of that organ. In frog's liver there is, as in muscle and nerve, an extra proteid coagulating at 36°-40° C. Here, however, the amount of shortening is less than in structures like muscle, nerve, and tendon, where the histological elements have a longitudinal direction.

The most interesting of these facts from a comparative standpoint is the coagulating temperature of the first proteid; because this is the temperature at which life is extinguished, and the electrical response, irritability, and conductivity abolished.

I have little doubt that a *prolonged* exposure to temperatures a few degrees below those given by Alcock would extinguish the electrical response in nerve, for the real coagulation process begins and occurs slowly then, as evidenced by slight shortening in experiments on heat contraction, and by opalescence in experiments with saline extracts.

The Coagulation Temperature of Cell-Globulin, and its Bearing on Hyperpyrexia

Results such as these we have been speaking of have not only an academic interest, but also a direct practical bearing for

the contraction due to the connective tissue is insignificant in amount; and on heating to 70° a further shortening occurs, just as in fractional heat coagulation of saline extracts of nervous tissue a proteid is found to coagulate at that temperature.

the pathologist. In carrying out work from this point of view, I have been associated with Dr Mott, and the following are our results and conclusions.

It is well known that there are various factors that influence the temperature of heat coagulation of proteid substances. Among these the rate of the rise of temperature is one of some importance. This was clearly demonstrated in the work of Corin and Ansiaux,* and of Hewlett.† These observers showed in connection with serum and egg-white respectively that if the temperature is maintained long enough below the point at which heat coagulation is usually stated to occur, not merely opalescence but the formation of flocculi will take place (see also p. 17).

In performing the process of fractional heat coagulation with extracts of various mammalian organs and tissues, I‡ have shown that in nearly all of them a proteid is present that coagulates at an extremely low temperature, which varies in different cases from 45° to 50° C. This proteid is a globulin, and has been variously named. Thus, in muscle we have learnt to call it para-myosinogen; in liver cells it has been called hepato-globulin; in extracts of nervous tissues, neuro-globulin; in extracts of lymph-cells, cell-globulin, and so on. There can be very little doubt that such a globulin is characteristic of protoplasmic structures, and even if it is not absolutely the same proteid in all cases, the term cell-globulin may be provisionally employed in a general sense to indicate that cells, as a rule, yield to saline solvents a proteid with characteristically low coagulation temperature.

One might, however, object that the behaviour of saline extracts of cells does not necessarily teach us the condition of the proteids as they are actually present in the complex we call protoplasm. In view of such a criticism, I attach special importance to the researches subsequently carried out by Brodie

* *Bulletin de l'acad. roy. de Belgique*, 1891, vol. xxi., p. 3.

† *Jour. of Phys.*, 1893, vol. xiii., p. 494.

‡ See Schäfer's *Text-Book of Physiology*, vol. i., art. "The Chemistry of the Tissues and Organs," by W. D. Halliburton.

and Richardson,* and later by Vernon.† We have already seen that these investigations show in the case of muscle that the shortening which occurs in the process of heat rigor is not a single one, but takes place in a series of steps; the temperatures at which these steps occur are the same as those at which the individual proteids separate out during the fractional heat coagulation of an extract of muscular tissue. Thus in mammalian muscle the two principal shortenings occur at 47° and 56° C., the coagulation temperatures of the two principal muscular proteids. In frog's muscle there are three steps at 40°, 47°, and 56° C. respectively, which correspond to the three proteids that can be separated out in a saline extract of this variety of muscular tissue.

I may also remind you once more that Brodie and Richardson showed another important point, namely, that after the first step has occurred in the shortening, the muscles lose their irritability; in other words, in order to destroy the vitality of muscular tissue, it is not necessary to raise the temperature sufficiently high to coagulate all its proteids, but that when one of the muscular proteids has been coagulated, the living substance as such is destroyed. It therefore appears to be the case that the proteids of muscle are not independent units. The unit is protoplasm, and if one of its essential constituents is destroyed, protoplasm as such ceases to exist.

These experiments in connection with muscle would lead one to suppose that the same is true in regard to other protoplasmic structures; that is to say, the results which have been obtained by the examination of saline extracts of such structures can be applied to the elucidation of the composition of the protoplasm of which they are composed.

This last sentence, which is word for word what I wrote a few years ago, has been abundantly justified by the experiments with nerve, which have formed the subject of the first two sections of this lecture.

* *Phil. Trans.*, 1899, vol. cxci. B., p. 127.
† *Jour. of Phys.*, 1899, vol. xxiv., p. 239.

Dr Mott's attention was directed to a consideration of this subject in connection with the question of hyperpyrexia. He found that the nerve-cells after death from this condition show a diffuse staining with methylene blue, and a disappearance or breakdown of the Nissl granules.

It is a familiar fact that very high body temperature is incompatible with life. Marinesco * has pointed out, in experiments on hyperthermia in mammals, that a temperature of 47° C. is immediately fatal; a temperature of 45° C. kills in an hour or two; a temperature of 43° C. kills after a longer lapse of time. Moreover, the occurrence of death is coincident with the breakdown of the nerve-cells in the manner just indicated. It is probable that analogous changes occur in other cells of the body also, but these have not yet been specially investigated. The nerve-cells are undoubtedly essential to healthy life, and lend themselves very readily to microscopic investigation, especially by the methylene-blue process. A temperature of 47° C. leads to an instantaneous disappearance of the chromatophile granules; the same change occurs at 45° C. in a few hours; at 43° C. a longer lapse of time is necessary.

We were struck with the coincidence of the fatal temperature (47° C.) with that of the coagulation temperature of neuroglobulin; and we argue that, as in muscle, the coagulation of even the lowest coagulating proteid of nerve-cells would produce a destruction of the life of their protoplasm; a distinct chemico-physical explanation can therefore be found for death due to hyperpyrexia.†

Still a temperature as high as 47° C. (117° F.) in man is unknown; and we thought it possible that the proteid in question would coagulate at a lower temperature if it was kept at that

* "Recherches sur les Lésions des Centres Nerveux consecutives à l'Hyperthermie Expérimentale et à la Fièvre," *Revue Neurologique*, 1899. See also Goldscheider and Flatau, *Norm. u. path. Anat. d. Nervenzellen*, Berlin, 1898.

† In cold-blooded animals, a far lower temperature is fatal, which is quite intelligible, seeing their tissues contain a proteid which coagulates below 40° C.

temperature a sufficient length of time. We proceeded to put the suggestion to the test of experiment, fully anticipating, in the light of the work of Hewlett and others, alluded to (p. 108), that the supposition would turn out to be correct. Experiment has shown that this is the case.

The first experiments were made with the brains of cats. After the animal had been killed by bleeding (sufficient chloroform having been given to render it unconscious), the brain was rapidly removed; the grey matter was finely minced and ground up in a mortar with 0.9 per cent. solution of sodium chloride. We selected this solvent as the one likely to produce least change in the constituents of the protoplasm. After repeated filtration, the extract remained somewhat opalescent; it was fairly rich in proteid, as tested by rapidly boiling a sample. It did not prove at all difficult to see any increase in the opalescence when the extract was carefully heated in a water-bath. The extract was faintly alkaline, but we judged it best not to add any acid to neutralise this, in order that we might deal with as natural conditions as possible.

When the rate of observation is fairly rapid, the first crop of flocculi was observed to separate out at 47° C. These are removable by filtration, and the filtrate is clear.

In our next experiment the temperature was not allowed to rise higher than 45°, and was kept between 44° and 45° C., being more frequently nearer the lower than the higher of these limits. In somewhat less than two hours the separation of flocculi took place, and as good a coagulum was ultimately obtained at this temperature as was obtained in the first experiment at 47° C. Previous to the formation of actual flocculi, there was an increase of opalescence, which became denser as time went on.

In the next experiment an attempt was made to obtain the coagulum at a still lower temperature, namely, 42° C. (108° F.); here again we were rewarded with success; there was at first the gradual deepening of the opalescence, and in time a distinct separation of minute flocculi, which increased in number and

size. The first separation of visible flocculi occurred about three hours after the commencement of the observation, and an hour later the crop was fairly abundant, though the size of the coagulum was not so great as in the previous two experiments. After filtering, the flocculi were, of course, removed, but the filtrate was still distinctly opalescent. Doubtless, if we had continued to watch the tube for a longer time the coagulation would have been more complete.

The next experiment consisted in trying a still lower temperature, namely, 40°-41° C. In this case, however, though the tube was watched for eight hours, there was no coagulation.

We have repeated this series of experiments several times, and in some cases, instead of grinding up the brain substance with salt solution only, we have employed clean sand or powdered glass as well. By this means one obtains an extract richer in proteid, filtration is easier, and the filtrate clearer. The phenomena of heat coagulation are exactly the same as in the experiments just described, but the proteid being more abundant, they are more readily seen.

In a further series of experiments we have employed human grey matter, removed from the cadaver as soon as possible after death. We have selected the optic thalamus as a convenient mass of grey matter for this purpose. The results absolutely agree with those already given.

We had hoped to have had an opportunity of similarly investigating the grey matter after death had supervened in hyperpyrexia; but since we began this work no such case came under our notice. We have accordingly had to be content with experiments on animals. A cat was, after anæsthetisation, rapidly killed by bleeding; the brain was removed as quickly as possible, and divided into two equal halves; this was first done roughly, and then the two halves were accurately made equal by removing fragments of the white matter from the heavier moiety. Each weighed about 9.5 grammes. One half was immediately ground up with powdered glass and normal saline

solution, and the extract examined. The other half was first heated to 47° C. for an hour, and then similarly treated, the same volume of saline solution being used. In the extract of the first (the normal) half, fractional heat coagulation revealed the presence of coagula, which came down at 47°, 56°-60°, and 72° C. respectively. In the extract of the second (the heated) half, the 47° coagulum was absent, but the other two were obtained. The total amount of proteid in the two extracts was also estimated in the usual way, by weighing the precipitate produced by excess of alcohol. 100 c.c. of the first (normal) extract contained 0.674 grammes; 100 c.c. of the second (heated) extract contained only 0.144 grammes of proteid material. The amount of cell-globulin which passes into solution in normal saline is thus relatively large.

In a second experiment, the half-brain was heated to 42° C. instead of 47° C. It was kept at 42° C. for five hours. Examination of the extracts showed that the extract of the normal half gave the usual crop of coagula, and 100 c.c. contained 0.483 grammes of proteid; the extract of the half-brain which had been heated to 42° C. gave as before no coagulum at 47° C.; 100 c.c. of this extract contained 0.226 grammes of proteid. The chemical examination of brain tissue as fresh as possible thus gave results which exactly correspond to those obtained in the experiments with saline extracts of brain.

The same is true for the histological examination we have made with "surviving" brain tissue.

We have not repeated Marinesco's experiments on hyperthermia in animals, but we have performed the experiment of exposing the brain *in situ* immediately after death to an elevated temperature. Two cats were anæsthetised and decapitated; the heads were placed in a warm chamber, a thermometer being inserted into the brain through the foramen magnum. In one case the brain was kept at 44° to 45° C. for one and a half hours; in the second, at 42° to 43° for three and a half hours. In each case, and particularly in the first one, the cells exhibited chromatolysis.

The accompanying photo-micrograph (Fig. 12) shows the appearance of the cells from a case of hyperpyrexia in a boy.

We may, therefore, sum up by saying that our experiments confirm our hypothesis, that the physico-chemical cause of death from hyperpyrexia is due to the coagulation of cell-globulin. When this constituent of cell-protoplasm is coagulated the protoplasm as such is destroyed. The temperature at which such coagulation is most easily produced is 47° C. But temperatures as low as 42° C. will have the same effect, provided the heating is continued long enough. These chemical changes in the brain substance are demonstrable by experiments with saline extracts of that tissue, or with the "surviving" brain of animals just killed. They are coincident with the histological (chromatolytic) changes in nerve-cells, which can be rendered evident by the use of the methylene-blue method. The expression coagulation necrosis employed by Marinesco for this appearance is, therefore, justifiable, though Marinesco and other histologists who have obtained similar results missed the connection of the temperature necessary to produce it, with that of the coagulation temperature of cell-globulin. Lastly, though the nerve-cells are those which lend themselves most readily to the histological part of the research, it is by no means improbable (looking at the wide distribution of cell-globulin), that many other cells of the body are affected by a high temperature in a corresponding manner.

It will be noticed throughout this part of our work, we were concerned with mammals only, because our object was to draw conclusions on the subject of hyperpyrexia in man. As was previously pointed out (p. 110, footnote), it is perfectly well known that cold-blooded animals, like frogs, are killed by a lower temperature than mammals, and this depends on the presence of the additional proteid in their tissues, which coagulates at 36°-40° C. In the process of evolution, when animals were formed higher in the scale which we term warm-blooded, and which maintain a normal temperature as high

FIG. 12.—Section of spinal cord from a case of hyperpyrexia, the temperature being 109° F. before death. The whole of the cells throughout the central nervous system showed a diffuse homogeneous staining with methylene blue. The Nissl granules had entirely disappeared from the processes and body of the cells, and the stainable substance had a fine, dust-like appearance. Magnification, 400 diameters.—MOTT.

[*To face page* 114.

as the death temperature of a frog, the cells adapted themselves to the altered circumstances, and this proteid of their protoplasm disappeared. Again, in birds, with a still higher normal temperature, there is a further adaptation, as pointed out on pp 104-107.

LECTURE IX

THE CHEMICAL PATHOLOGY OF CERTAIN DEGENERATIVE NERVOUS DISEASES

I PROPOSE in the present lecture to deal with more strictly pathological questions. The new subject of chemical pathology has a great future before it, and this is as true for nervous diseases as for diseases of other parts of the body. Up to the present time comparatively few pathologists have worked at the chemical side of nervous disease; and a few years ago, when Mott and I started our work, this branch of investigation was almost untouched.

Chemical Pathology of General Paralysis of the Insane

The first disease which attracted our attention is the very common one known as General Paralysis of the Insane; and I will first deal with our results in that disease, because it is a typical example of a degenerative disease, in which there is considerable destruction of nervous tissue. It is a para-syphilitic affection like tabes, with which it is, pathologically speaking, identical. It is a premature, primary, progressive decay of the neuron, affecting especially those structures which have been developed latest. To the naked eye the extensive, degenerative, and wasting process which occurs, especially in the frontal and central convolutions, is perfectly evident. Microscopical examination of the diseased brains reveals degenerative changes in the cells; and the perivascular lymphatics are seen (by Marchi's method of staining) in acute cases to contain phagocytes filled with black-stained fatty matter. During the

course of the disease there are seizures of an epileptiform or apoplectiform kind, and after recovery of the patient from each of these fits he is, as a rule, worse mentally. Each fit indicates the breakdown of a new focus of cerebral matter.

The place of the atrophied brain substance within the cranium is taken by excess of cerebro-spinal fluid. It is often possible to obtain as much as 100 to 200 c.c.

The main object of our research was to examine the cerebro-spinal fluid, and to attempt to discover in it some substance or substances derived from the disintegration of the brain-matter, which, passing thence into the general circulation, would produce auto-intoxication, and thus account for some of the symptoms of the disease.

Normal cerebro-spinal fluid is alkaline to litmus, and contains a very small percentage of solids (see first Lecture). The fluid from cases of General Paralysis is not only more abundant, but is also richer in solids; this is principally due to excess of proteid material. The average percentage of proteid in eight specimens was 0.24, that is about three times as much as is found in cerebro-spinal fluid in cases of spina bifida. It is alkaline, like the normal fluid. Fibrinogen is absent, as in the normal fluid; proteoses and peptone are also absent. There is a small quantity of albumin present; in the normal fluid albumin is absent. The most abundant proteid, however, is nucleo-proteid; in one case sufficient was present to produce intravascular coagulation, when 10 c.c. of the fluid were injected into the jugular vein of a cat.

Another distinction between the normal and the pathological fluid is the presence in the former of a reducing substance, and the absence of this, as a rule, in the latter; in only two out of fourteen cases was it found.*

But the most noteworthy distinction between the two fluids is the presence of abundance of choline in the specimens removed from cases of General Paralysis. The chemical examination of the fluid supports our contention that the cerebro-spinal fluid acts as the lymph of the brain, and when the disintegration

* This may possibly have been due to glycolysis, as all these fluids were removed from the cadaver.

of the cerebral tissue is increased, as in General Paralysis, the fluid contains the products of such disintegration (*e.g.*, choline, nucleo-proteid). The greater number of the specimens of cerebro-spinal fluid we examined were removed from the cadaver as soon as possible after death. We also had the opportunity of examining four specimens removed during life by lumbar puncture from cases of General Paralysis.* The results obtained with these are identical with those obtained from the post-mortem specimens. We have also secured on several occasions blood removed for remedial purposes from such patients by venesection, and we regard one of the most important outcomes of our work, the discovery that the blood also contains the same toxic material during a seizure.

Let me now trace the way in which we were led to identify the substance in cerebro-spinal fluid as choline.

We found that the cerebro-spinal fluid itself when injected into the blood stream of a living anæsthetised animal (rabbit, cat, or dog) produced a fall of blood pressure, whereas normal cerebro-spinal fluid has no such effect. We at first thought it was the proteid material in the fluid which was responsible for this result. But we found that the removal of the proteid by heat or by alcohol makes no difference in the observed result. Our next supposition was that the active substance might be inorganic. We therefore took a large quantity of the fluid, evaporated it to dryness, and incinerated the residue. The ash was dissolved in physiological salt solution, and injected with a negative result. The active substance is therefore of organic nature, but is not proteid. We then took the alcoholic filtrate of the fluid, evaporated off the alcohol at 40° C., took up the solid residue with absolute alcohol, filtered it, and again evaporated off the alcohol. This was repeated two or three times more, in order to lessen the danger of any potassium salts remaining in solution. The final residue was dissolved in physiological saline solution; we injected this, and obtained a fall of pressure like that produced by the original fluid. The active substance is therefore of organic nature, and one which

* We were indebted for these to Dr John Turner of the Essex County Asylum.

is soluble both in alcohol and in physiological saline solution. It then occurred to us that the substance might be alkaloidal; we accordingly took a solution of it in saline solution prepared as just described, and added to it phospho-tungstic acid in the presence of sulphuric acid until no more precipitate occurred. We then took both precipitate and filtrate, and from the former separated out a base which produced a fall of blood pressure; whilst from the latter (the filtrate) no such material was obtainable. We naturally next thought of the bases which would most likely be present; the base which we first thought of was choline. It accordingly became necessary to make an examination of the physiological action of pure choline, to which we added a corresponding examination of the action of the closely related base neurine.

At the same time, it was necessary for us to identify choline and neurine by means of chemical tests.

We will first take the physiological action of the two bases, and then their chemical reactions. We may anticipate the result by saying that the substance in cerebro-spinal fluid proved to be identical in its action, and in its chemical behaviour, with choline. Neurine is not present.

Physiological Action of Choline and Neurine

We injected quite small quantities (1 to 5 c.c. of a 0.2 per cent. solution either of choline itself or of its hydrochloride), for we sought as far as possible to note the effects produced by solutions of such strength as would be comparable to the amount of the base presumably present in pathological cerebro-spinal fluid. Its actions are briefly as follows:—

(1) Choline produces a temporary fall in arterial blood-pressure.

(2) This is in some measure due to its action on the heart.

(3) It is also, and probably mainly, due to dilatation of the peripheral vessels, especially in the intestinal area.

(4) The kidney and limbs undergo a passive lessening of volume secondary to the fall in general arterial pressure.

(5) The spleen contracts markedly, and when this passes off, its normal curves are greatly exaggerated.

(6) We obtained no evidence of any direct action of the base on the cerebral vessels.

(7) The action on the splanchnic vessels is due to the direct action of the drug on the neuro-muscular apparatus of those

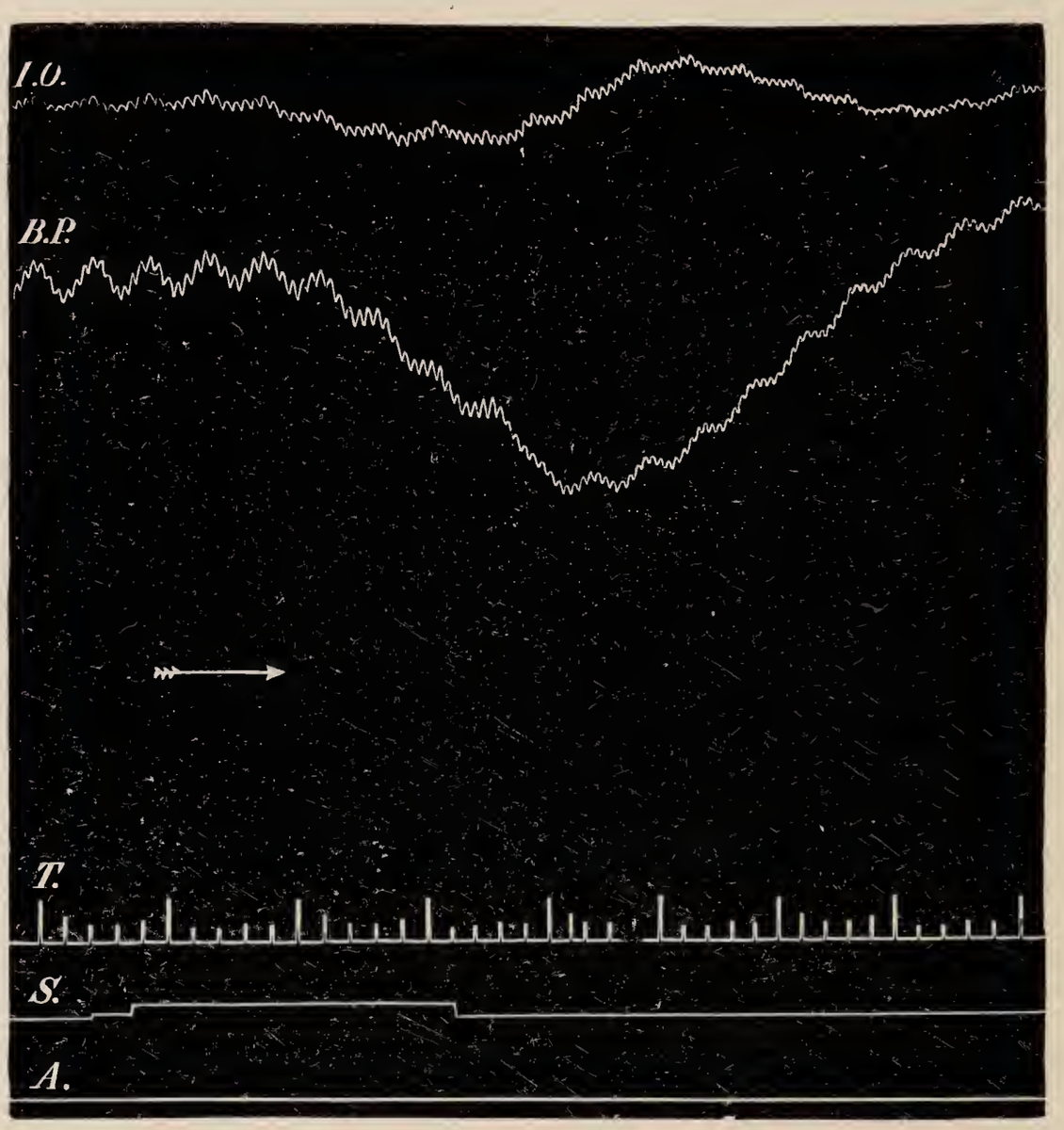

FIG. 13.—Original size. Tracing of intestinal oncometer (*I.O.*) and arterial blood pressure (*B.P.*) in a cat; 10 c.c. of cerebro-spinal fluid from a case of General Paralysis were injected; the same effect was obtained in the same animal by injecting 2 c.c. of a 0.2 per cent. solution of choline. The fall of blood pressure is at first mainly cardiac in origin, for the oncometer tracing first follows the fall of arterial blood pressure passively; it, however, soon rises, indicating dilatation of the peripheral vessels.

vessels, for after the influence of the central nervous system has been removed by section of the spinal cord, or of the splanchnic nerves, choline still causes the typical fall of arterial

pressure. The action of peripheral ganglia was in other experiments excluded by previously poisoning the animal with nicotine.

(8) Choline has very little effect on the galvanometric response of nerve-trunks; neurine has a depressing effect (Waller and Sowton).

(9) There is no effect on the respiration.

(10) Section of the vagi makes no difference in these experimental results.

(11) Previous atropinisation of the animal causes a great difference; it abolishes the fall of arterial pressure, though there is still some dilatation of splanchnic blood-vessels. In fact, very often injection of choline after atropine produces a rise instead of a fall of blood pressure.

This last point has already been illustrated by the tracings in Figs. 4 and 5 (p. 84).

In all these particulars choline and the basic substance separated out from the cerebro-spinal fluid or blood of these patients are in complete agreement. How close the agreement is can be seen by the numerous tracings reproduced in our Royal Society paper.

At the actual lecture many of these tracings were shown as lantern slides. I will be content here with giving one very typical tracing (Fig. 13), which shows the fall of pressure, and the accompanying vaso-dilatation as recorded by Edmund's intestinal oncometer.

It is obviously impossible with any one specimen to perform all the physiological tests enumerated under the foregoing eleven heads. The most readily available test, and which I shall subsequently speak of as *the* physiological test, is the fall of blood pressure with accompanying intestinal vascular engorgement. The animal next receives a small dose of atropine, and a further injection of choline then produces either no fall or it may be a rise of blood pressure instead.

This important test was demonstrated on an anæsthetised dog at the lecture.

It is unnecessary to enter into full details of the physiological

action of neurine, as it was never found in the cerebro-spinal fluid or blood of these patients. Suffice it to say that it is

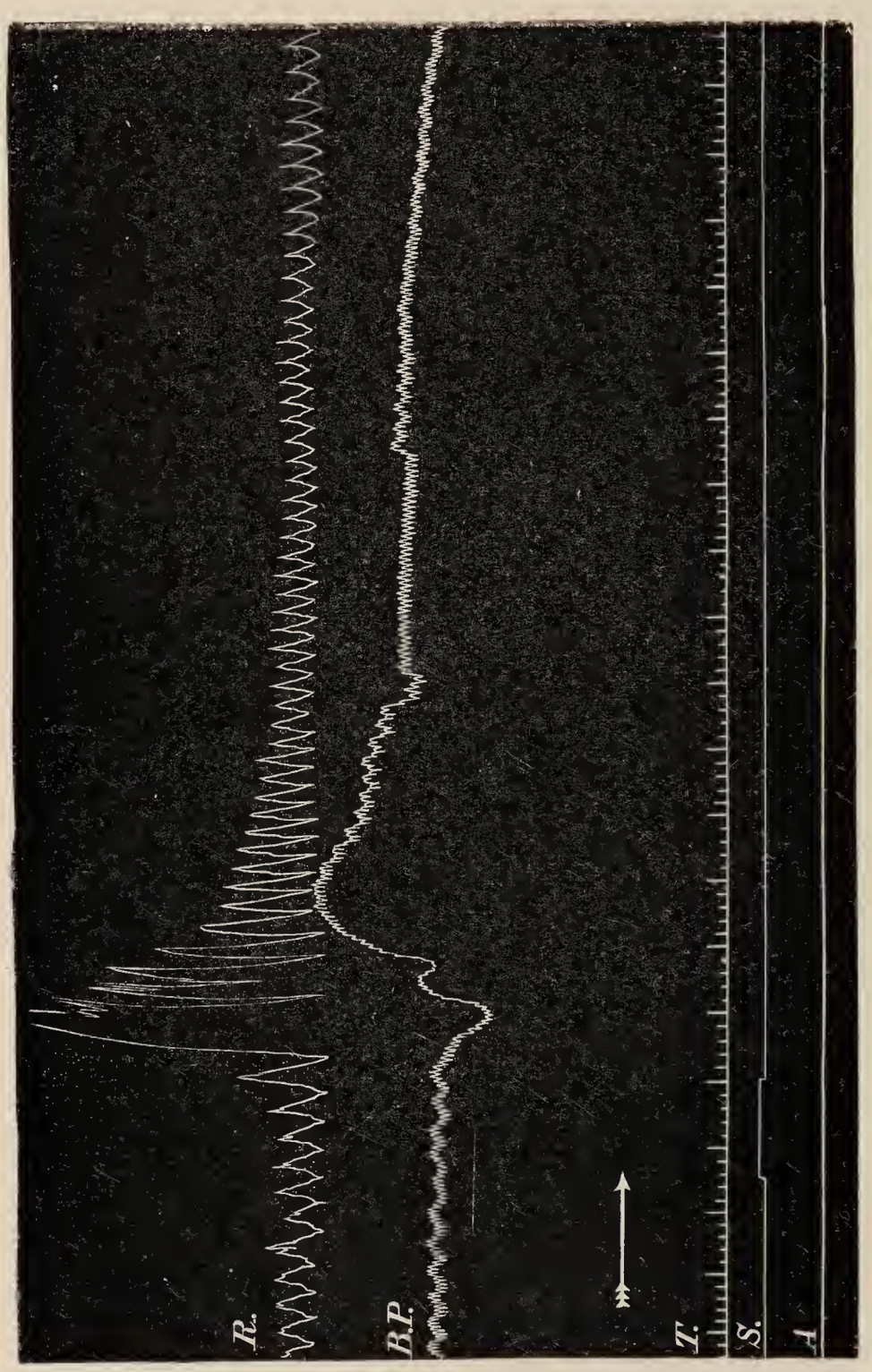

FIG. 14.—Half the original size. Effect of injecting in a cat 2.5 c.c. of a 0.1 per cent. solution of neurine. In the tracing (*R.*) each upstroke is caused by inspiration. Note the increase in respiratory efforts followed by a decrease. In the blood pressure tracing (*B.P.*) there is a fall followed by a pronounced rise.

much more poisonous, it affects the heart more (and the effect on the heart is neutralised by atropine), causes peripheral constriction rather than dilatation of the peripheral vessels,

stimulates and then paralyses the respiratory movements, and has a curare-like action on the voluntary muscles.

It will be sufficient to reproduce one tracing of the action of neurine. I have selected one which shows the effect on arterial pressure and respiration (Fig. 14). I have inserted it to show that there can never be any confusion in the physiological test between choline and neurine.

Chemical Reactions of Choline and Neurine

The repeated treatment with absolute alcohol alluded to in the method we adopted of separating out the base from the cerebro-spinal fluid and blood of these patients, had for its object the removal of proteid, and of salts of ammonium and potassium. In order to ensure as little admixture of these inorganic salts as possible, it is essential that the alcohol used should be as water-free as possible. The final residue was crystalline at first, but soon deliquesced on exposure to air. It is soluble in water, physiological saline solution, alcohol, and ether. On putrefaction it gives off the odour of ammonia and trimethylamine. Dissolved in water or in physiological salt solution, it gives white precipitates with phospho-tungstic acid, phospho-molybdic acid, and mercuric chloride. It gives a brownish precipitate with iodine dissolved in a solution of potassium iodide or in alcohol, a yellow precipitate with gold chloride, and with platinum chloride. The alcoholic solution gives with gold chloride a precipitate which consists of tiny yellow crystals which are soluble in hot water and hot alcohol; they are insoluble in ether.

In all these points the base from the cerebro-spinal fluid or blood exactly resembles choline.

The aqueous solution gives no precipitate with tannin, which distinguishes it from neurine. The two bases are also readily distinguishable by means of their chromates, that of neurine being scarcely soluble, while that of choline is readily soluble in cold water (Cramer *).

* *Jour. of Phys.*, 1904, vol. xxxi., p. 30. Cramer also found no neurine from the decomposition of protagon.

In my experience, the most readily applicable and the most delicate of these chemical tests is the platinum test.* An alcoholic solution of platinum chloride is added to an alcoholic solution of choline hydrochloride. The precipitated platino-chloride is of a yellow colour, easily soluble in water, and so is distinguishable from the platino-chloride of ammonium and of potassium. It is insoluble in ether, and readily soluble in 15 per cent. alcohol.

On evaporating the solution in 15 per cent. alcohol to dry-

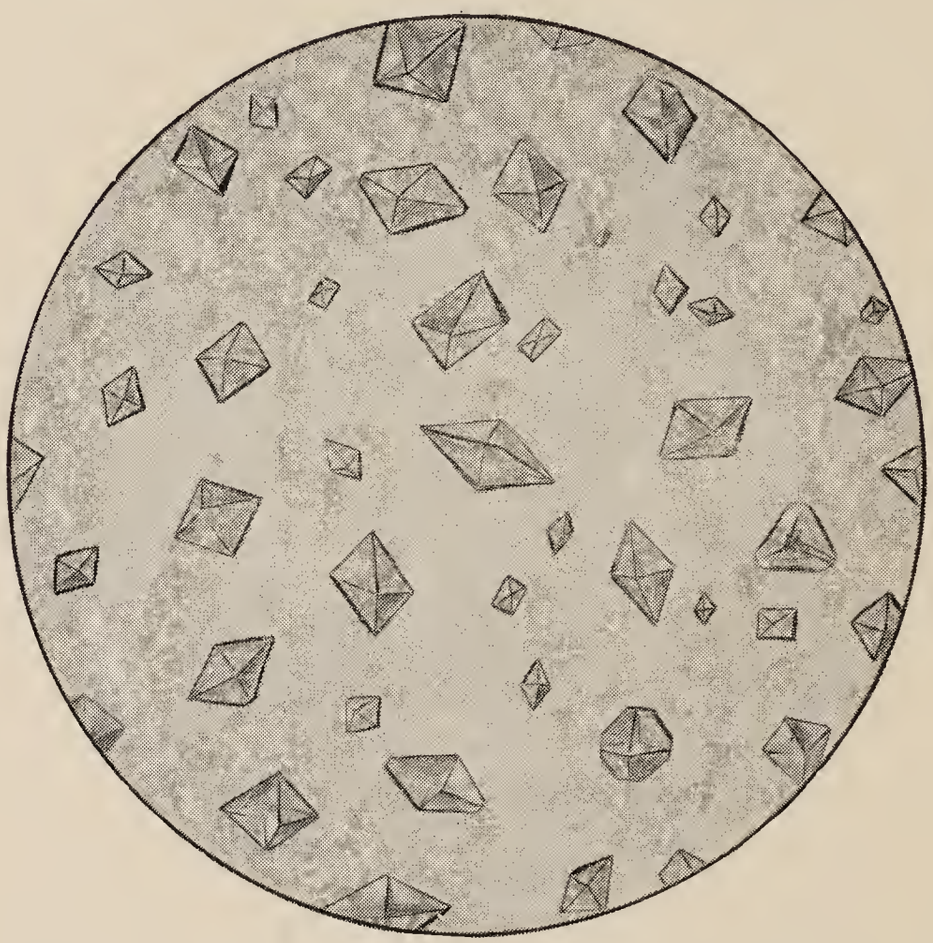

FIG. 15.—Crystals of the platino-chloride of choline prepared from a solution of choline hydrochloride. Crystallised from 15 per cent. alcohol.

ness at a low temperature (40° C.), it crystallises out in yellow octahedra, shown in the accompanying drawings.

It is always dangerous to attempt to identify any substance merely by its crystalline form. It is well known that the platino-chlorides of potassium, of ammonium, and of bile salts crystallise in a very similar way. So in cases where there was

* See also Gulewitsch, *Zeit. f. physiol. Chem.*, vol. xv., p. 149.

sufficient material to work with, we further examined the characters of the choline platino-chloride, and found that its solubilities, the amount of platinum it contains, and the fact that on heating it gives off trimethylamine, are sufficient to definitely distinguish it from the others mentioned.

When the platino-chloride of choline is dissolved in water and allowed to crystallise, the crystals formed are six-sided

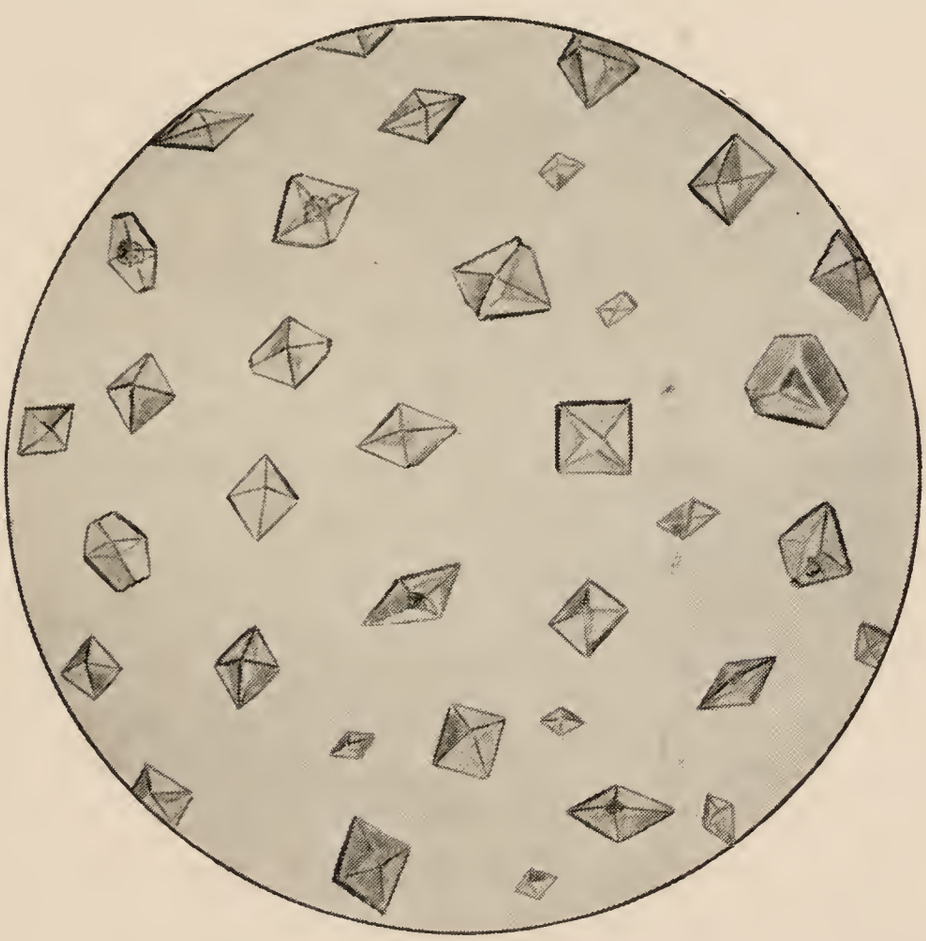

FIG. 16.—Crystals of the platino-chloride of the base separated from cerebro-spinal fluid in cases of General Paralysis of the Insane. Crystallised from 15 per cent. alcohol. The identity of this base with choline is not only shown by the form of these crystals, but by its solubilities and by other numerous reactions described in the text.

plates and needles of a yellow colour. These usually form curious aggregations described by Donath * as star-fish crystals, with four rays, one usually much larger than the others. These crystals are regarded by Donath as more typical than the octahedra Mott and I figured, and form an even better microscopic chemical test for small amounts of choline.

* *Zeit. f. physiol. Chem.*, 1903, vol. xxxix., p. 526.

I may sum up the main results which we obtained in cases of General Paralysis in the following way.

The cerebro-spinal fluid of patients suffering from the disease contains toxic material, which originates from the disintegration of nervous tissues mainly in the brain. We obtained chemical evidence of the existence of nucleo-proteid in the fluid, and though the amount of nucleo-proteid is not, as a rule, sufficient to cause massive intravascular clotting when the fluid is injected into animals, we consider that the presence of even small quantities continuously being poured out into the cerebro-spinal fluid, collecting in the perivascular lymphatics and passing thence to the blood, will produce harmful results. The idea suggested itself to our minds that an increase in the coagulability of the blood in the small vessels of the cerebral region, which nucleo-proteid would produce, might form a determining factor in promoting venous stasis, and thus are caused the acute manifestations or seizures of apoplectiform or epileptoid nature which the patients exhibit.

The other toxic substance which we succeeded in isolating is choline. The existence of choline in the cerebro-spinal fluid and also in the blood, is a clear indication of the disintegration of the brain tissue. Choline, however, is not a very poisonous substance. Glycero-phosphoric acid and lactic acid are even less toxic.

Still, even choline has some effect. A feeble circulation and fatty degeneration of the heart are very frequent concomitants of the terminal stages of the disease, especially after a series of seizures, and the idea seems feasible that choline may in part explain these. A single dose of choline in a dog or cat produces but little effect on the heart; still there is some effect, and it does not appear a far-fetched idea to suppose that the continual pouring of small doses of choline into the cardiac tissue may in time produce cardiac weakness, and even degeneration.

After prolonged convulsions, the blood pressure of these patients (as tested by the Hill-Barnard sphygmometer) falls considerably; it rises again a few days after the convulsions have ceased. There can be no doubt that the convulsions are

associated with the breaking down of nervous tissue, and we therefore think it probable that the choline so liberated is responsible for the fall of pressure which occurs then.

Whether choline will explain the fits themselves, is another question. We were never successful in producing anything like a fit in our experiments on anæsthetised animals, even when we injected quite strong doses of the base into the carotid artery. Donath, however, has stated that by injecting the material into the cerebral substance in the region of the sensori-motor convolutions, he has produced convulsive attacks in animals. If this is confirmed it is an indication that choline is a more toxic material than Mott and I considered it to be.

Cholinein the Blood, and Cerebro-Spinal Fluid in other Degenerative Diseases of the Nervous System

We have now seen that in the disease called General Paralysis of the Insane, the degenerative changes that occur in the central nervous system are associated with the presence of the products of such degeneration in the cerebro-spinal fluid. One of these products, choline, is derived from the breakdown of lecithin. Choline can be identified in the blood also of these patients. The tests on which one relies for the detection of this alkaloid are mainly two: the first is a chemical test, namely, the obtaining of the typical yellow crystals of the platino-chloride from the alcoholic extract of the blood. These crystals have not only a definite form, but their solubilities distinguish them from other somewhat similar crystals, as also does the fact that they yield a fixed percentage of platinum, and give rise to an odour of trimethylamine when decomposed by heat. The second test is a physiological one: a saline solution of choline, of choline hydrochloride, and of the residue obtained from the alcoholic extract of the cerebro-spinal fluid and blood of these patients, produce a temporary fall of pressure when injected intravenously in animals. This fall is partly cardiac in origin, and partly due to dilatation of peripheral blood-vessels; the dilatation is due to the direct action of the alkaloid on the neuro-muscular mechanism of the blood-vessels themselves.

There are many substances which produce a fall of arterial pressure, but choline is peculiar in the fact that after the administration of a small dose of atropine, it no longer produces a fall but a rise of blood pressure, or, at any rate, the fall is abolished.

In the investigation of the blood, as a rule, only a small amount of material has been at one's disposal, and in order to obtain satisfactory evidence of choline it is necessary to considerably concentrate the alcoholic extract. It is therefore necessary to limit the number of tests to be performed. The two tests, however, appear to be, if positive, quite conclusive evidence of the presence of choline.

The only other organic substance with which I am acquainted which gives the physiological test is spermine,* but the chemical reactions of this substance are so different from those of choline that there can be no risk of confusing the two.

Bile salts, which Croftan † has shown to be present in blood, give a platino-chloride of similar crystalline form and colour to that given by choline when the crystallisation takes place from 15 per cent. alcohol; but when crystals are obtained by the evaporation of an aqueous extract, as in Donath's method, the peculiar star-fish crystals so typical of the choline compound are never obtained (O. Grünbaum ‡). In the matter of the physiological test, solutions of bile salts do produce a slight fall of arterial pressure; but quite strong solutions (5 per cent.) are necessary to produce the result, and this effect is not abolished by atropinisation.

Solutions of potassium chloride and of ammonium chloride produce a fall of blood pressure, but again this is not abolished by atropine. The similarity of the platino-chlorides has been already alluded to. Contamination with these inorganic substances may be largely excluded by the use of absolutely water-free alcohol, and if they are present the choline platino-chloride can be separated out by dissolving it in water, and

* Dixon, *Jour. of Phys.*, vol. xxv., p. 356.

† *American Jour. of Med. Science*, 1902, p. 150.

‡ Private communication to the author.

recrystallising; it then shows the star-fish crystals previously alluded to.*

The difficulty of obtaining cerebro-spinal fluid during life led Mott and myself in our later work to devote more particular attention to the blood; unfortunately, choline does not pass into the urine, so the withdrawal of a small quantity of blood from the patient is necessary. Ten c.c. of blood will give the tests in a marked case. This is a point of some practical importance; in cases where it is difficult to distinguish between serious cases of organic disease and cases of so-called functional neurosis, the performance of the tests described may come to the assistance of the practical physician in making his diagnosis.

FIG. 17.—Nerve degeneration in alcoholic neuritis; stained with osmic acid (S. MARTIN).

Normal blood used in these quantities gives negative results. If the platinochloride is crystallised from alcohol, a certain number of octahedra may be found; these may be due to small quantities of choline, but are more probably due to contamination with potassium and ammonium chlorides. The same is true for ordinary dropsical effusions.

General Paralysis is not the only disease where there is disintegration of nervous tissues; it was, therefore, to be anticipated that choline would be discoverable in the blood in other nervous diseases, and we identified it in many. Some of these are diseases of the central nervous system (disseminated sclerosis, combined sclerosis, etc.);

* The want of care in the use of *absolute* alcohol will probably explain most of the criticisms on the methods which have been made by Vincent and Cramer (*Jour. of Phys.*, vol. xxx, p. 143), and by Allen and French (*Proc. Phys. Soc.*, 1903, p. xxix; *Jour. of Phys.*, vol. xxx.). More lately, Allen has found that the iodine test for choline can be employed without any danger of confusion with inorganic chlorides. By means of this test, he has confirmed our statements on the presence of choline in the blood of patients suffering from extensive degeneration in nervous tissue (*ibid.*, xxxi., p. lvi.)

some of the peripheral nervous system (Beri-beri and other forms of neuritis). The degeneration of peripheral nerves in alcoholic neuritis is shown in the accompanying drawing (Fig. 17).

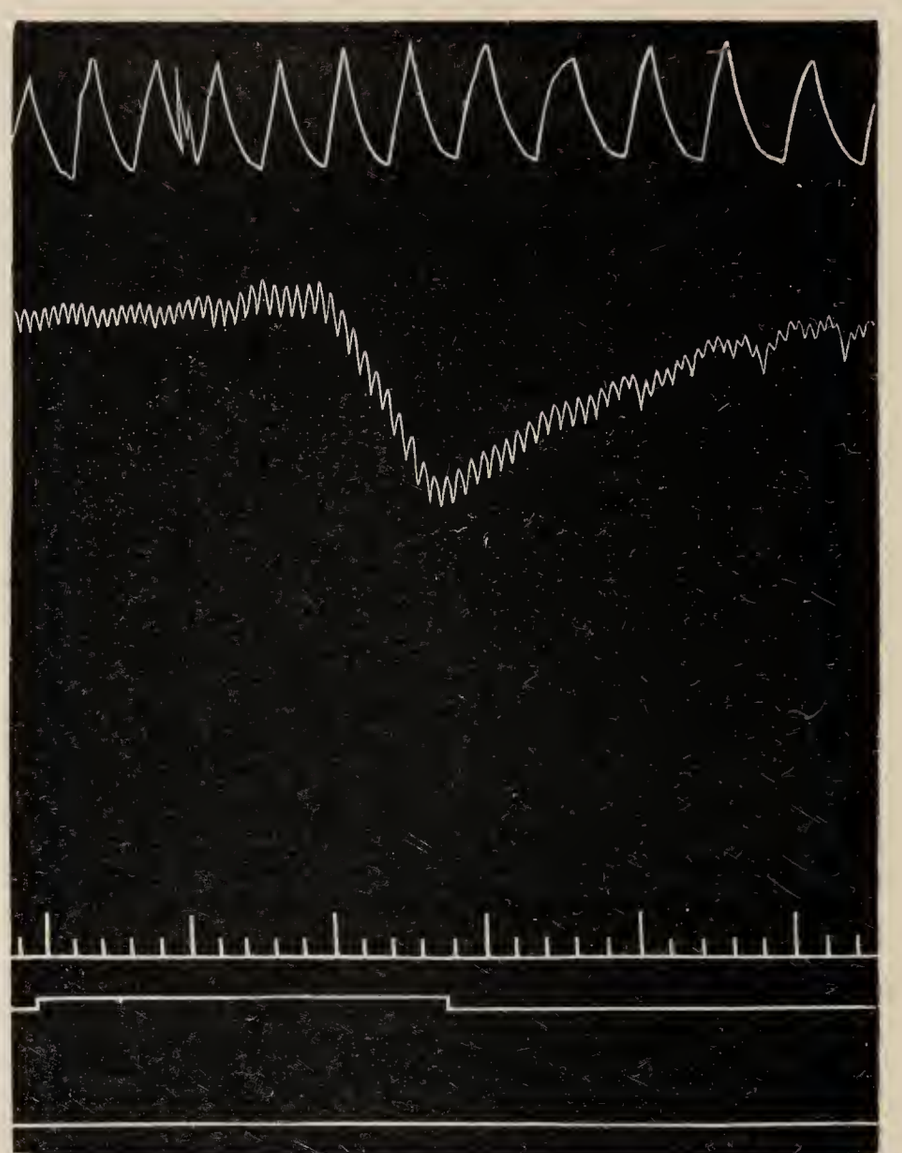

FIG. 18.—The uppermost line represents the respiration, taken by the tambour method. The next line is the blood pressure from the carotid of a cat. The next is a time-tracing in seconds ; the next is the signal line, the raising of which indicates the period of the injection. The lowest line is the abscissa of the blood pressure.

The injection produced as usual no effect on respiration. The actual volume of saline solution of the active material was 5 c.c. This was injected into the external jugular vein.

FIG. 18 represents the fall of arterial pressure produced by the injection into the external jugular vein of the choline obtained from 10 c.c. of the blood in a case of Beri-beri. This tracing is of the original size.

We found that the two tests fitted together with great accuracy. If the chemical test is performed first, one can

prophesy, from the amount of crystals, the result of the injection. If, on the other hand, the physiological test is performed first, one can prophesy accurately from the fall of blood pressure whether an abundant or scanty crop of crystals will be obtained.

The graphic records we have of these physiological experiments are very numerous, but the two given in Figs. 18 and 19 will serve as a typical sample. The case selected is one of Beri-beri.

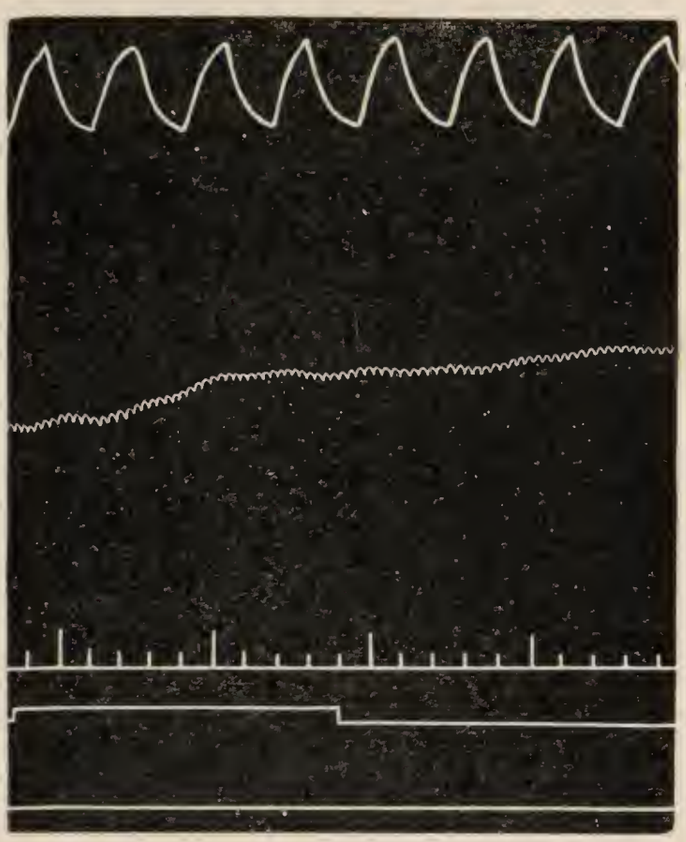

FIG. 19.—The result of injecting the same volume of the same solution in the same cat after atropine had been administered. There is now a rise of blood pressure.

Since our joint work was finished on this subject, Dr Mott has made further investigations on the value of the choline test (*i.e.* the chemical test), for active degeneration of the nervous system,* and concludes that the test is of no use to decide whether a case is organic or functional unless the organic disease is active at the time the blood is drawn; it is therefore specially applicable after the onset of new symptoms indicative of irritative or destructive processes.

Since our papers were published several other investigators have applied themselves to the same problem, and in spite of

* *Archives of Neurology*, vol. ii., p. 858.

the criticisms previously alluded to, the main results confirm our original statements.

Gumprecht* examined cerebro-spinal fluid, and found minute traces of choline in the normal condition. This is enormously increased, not only in General Paralysis but in many other diseases of the nervous system. He directed his attention in particular to acute diseases like meningitis.

Donath† also worked mainly with cerebro-spinal fluid, but made also a few observations on the blood. The cerebro-spinal fluid was obtained by lumbar puncture during life. He introduced some new details into the method adopted, so as to make the test more certain. He invariably finds choline in organic nervous diseases, and gives a long list of the cases he examined. It is absent in functional diseases. It is interesting to note that in one of the cases of neurasthenia he examined, the test was positive. This suggests either an incorrect diagnosis; or, if the diagnosis was correct, neurasthenia is not always a purely functional disorder.

Dana and Hastings‡ have examined the cerebro-spinal fluid of a large number of cases mainly from the point of view of cyto-diagnosis, but in two cases of alcoholic psychosis, where they instituted also a search for choline, they found it.

Otto Grünbaum has, more recently still, investigated the question, and, as this has been done at a time after the appearance of the criticisms of Allen and French, and of the suggestions of Donath, it may be regarded as the most satisfactory series of experiments yet made. His results with the chemical test applied to blood are as follow:—

In normal blood, choline is absent or present only in negligible quantities.

In four cases of herpes zoster, where degenerative changes are slight, the result was uniformly negative. The degenerative change must, therefore, be fairly extensive to get a positive result. In four cases of hysteria, and one case of tobacco poisoning, the result was also negative.

* *Verhandl. der Congr. für innere Med.*, Wiesbaden, 1900, p. 326.

† *Zeit. f. physiol. Chem.*, 1903, vol. xxxix., p. 526.

‡ *Medical Record*, New York, 23rd January 1904.

He obtained positive results in three cases of disseminated sclerosis, in two of paralysis agitans, in three of tabes dorsalis, in one of progressive muscular atrophy, in two of transverse myelitis, and one of myasthenia gravis.

In one case of progressive muscular atrophy, in one of disseminated sclerosis, and in one of transverse myelitis, he obtained negative results; but this really does not militate against the general conclusion, for in all three cases the disease had reached a quiescent stage.

In a second paper just published by Donath,* he has extended his work on the cerebro-spinal fluid, and shown that the amount of phosphoric acid in it is increased also when the degenerative lesion is sufficiently great. This is what one would expect, for phosphoric acid, like choline, is a product of lecithin decomposition. In functional conditions like epilepsy, melancholia, and hysteria, this increase of phosphoric acid does not occur.

Lecithin is a substance which should be also interesting to the pathologist from quite a different point of view. Preston Kyes † has advanced the view that it may play the part of the complement in toxin poisoning, *e.g.* in the case of snake venom.

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* *Zeit. f. physiol. Chem.*, 1904, vol. xlii., p. 141. In the same number of this journal (p. 157), G. Mansfield criticises Donath's researches upon choline. I have no doubt he will be able to satisfactorily dispose of these criticisms.

† *Ibid.*, vol. xli., p. 273.

LECTURE X

DEGENERATION AND REGENERATION OF NERVES

If one takes any animal cell, such as an amœba, and divides it into two parts, one part possessing the nucleus, and the other not, the former continues to live and thrive, the latter degenerates and dies. This general truth concerning the importance of the nucleus in regulating the nutrition of the cell receives special importance when applied to the units of the nervous system, because the fact that axons degenerate when cut off from the bodies of the cells of which they are outgrowths, has furnished physiologists with one of their most valuable methods of tracing and differentiating tracts of nerve-fibres. The degeneration of the distal segment of the nerve-fibre is a rapid and marked change, known after its discoverer as *Wallerian degeneration.* The slower atrophic changes in the proximal segment which take place if regeneration does not occur, are known as *disuse atrophy;* under these circumstances, the chromatolytic changes in the protoplasmic body of the cell is evidence of the relative inactivity of the cell-body.

The microscopic appearance of degenerated nerve-fibres is well known, and Mott and myself, approaching the subject from the chemical point of view, have sought to compare and correlate the chemical changes that occur with these histological alterations of structure.

For this purpose, we took a series of cats, and divided both sciatic nerves in the upper part of the thigh. The animals were killed at varying intervals after this operation, their blood was collected, and the nerves themselves examined both histologically and chemically. The histological method principally

employed was the Marchi reaction; our main object of examining the blood was to ascertain whether choline was present, and the macro-chemical methods in the examination of the nerves consisted chiefly in ascertaining the proportion of water and solids, and the amount of phosphorus in the solids.

Experiments with the Blood

These may be very briefly stated; the methods used were the same as those already described in connection with human blood.

The blood of normal cats contains the merest traces of choline. A few crystals can generally be found by the platinum test, though it is quite possible, in view of recent criticisms, that some or all of these crystals may have been due to a slight contamination with chlorides of potassium or ammonium. The amount of these crystals was too small to admit of a more detailed examination of them. If choline is present, it occurs in too small an amount to give the physiological test when 20 to 30 c.c. of the blood are employed.

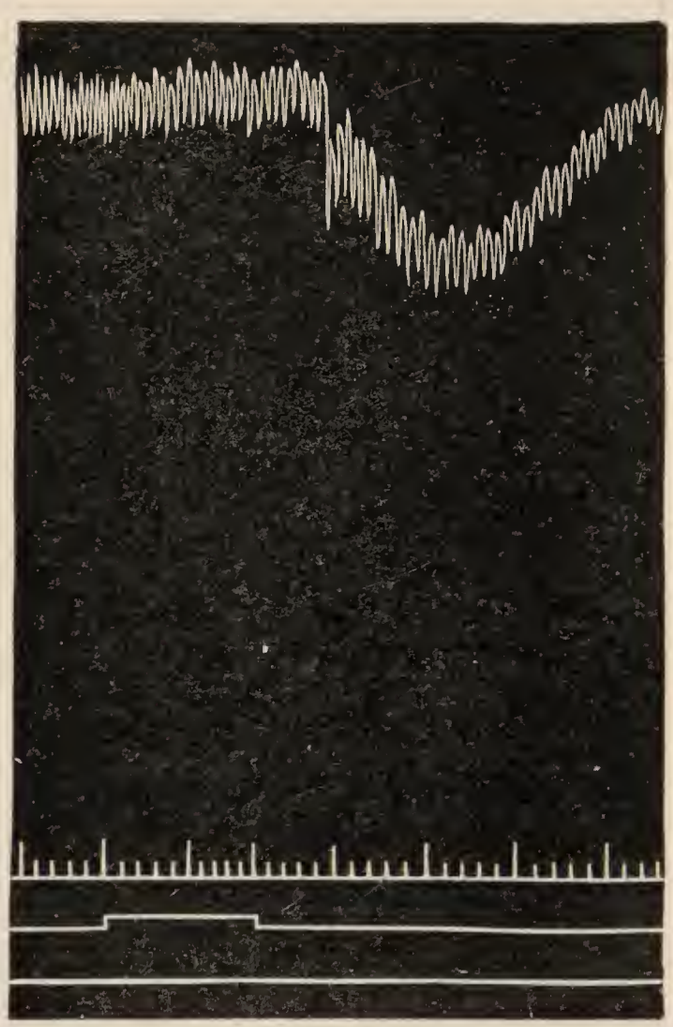

FIG. 20.—Result of injecting choline obtained from 30 c.c. of blood of a cat eight days after section of both nerves.

When signs of degeneration set in, evidence of chemical breakdown of lecithin was found in the presence of choline in the blood; it was detectable three or four days after the operation of cutting the nerves had been performed. The best yield

of crystals, and the most marked fall of blood pressure was in the case of the eight-day cat. By this time the Marchi method showed pronounced histological degeneration. This fall was abolished by atropine (see Figs. 20 and 21).

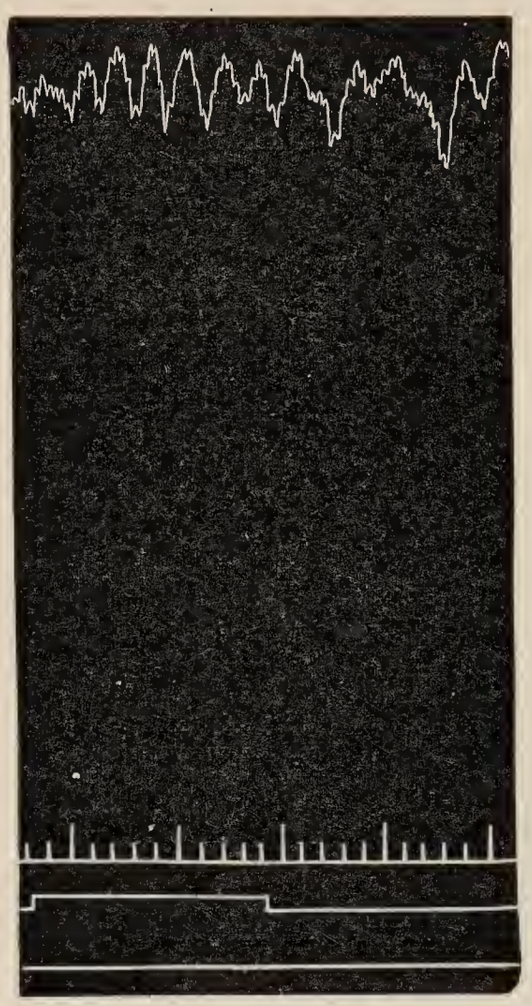

FIG. 21.—Result of injection of the same amount after atropine.

In the blood of the cat killed thirteen days after the operation, there was a good positive result, but there was less choline present; from this time onward the evidence of choline steadily diminished, until the normal was reached in the later stages of the degenerative process.

Chemical Examination of the Nerves

The nerves were carefully dissected out, weighed, dried to constant weight at 110° C., and again weighed. The dry residue was used for the determination of phosphorus. Considering the small weight of the nerves, we judged this would give more accurate results than any attempt to isolate the fatty material, and determine the phosphorus in that.

The dried nerve was soaked in 5 per cent. hydrochloric acid for three weeks, in order to get rid of inorganic phosphates. Such treatment apparently gets rid of the phosphorus combined as nuclein or nucleo-proteid; for in many of the nerves of later date there was considerable nuclear proliferation seen microscopically, and yet little or no phosphorus was obtained from the nerves after treatment in this way with hydrochloric acid. The phosphorus we did obtain came, therefore, either wholly or chiefly from the phosphorised fat. The nerves were then dissolved on the water-bath at 100° C. in fuming nitric and sulphuric acids, to which an occasional pinch of potassium

chlorate was added. The heating with acid was continued for many hours. The phosphate so formed was precipitated by ammonium nitro-molybdate. The yellow precipitate so obtained was washed, and dissolved in dilute ammonia, then precipitated by magnesia mixture; this precipitate was incinerated and weighed as magnesium pyrophosphate,* from which the amount of phosphorus was calculated.

The following table gives the main results; particulars are added relating to the choline in the blood, and the histological condition of the nerves.

Days after section.	Cat's Sciatic Nerves.			Condition of Blood.	Condition of Nerves.
	Water.	Solids.	Percentage of Phosphorus in Solids.		
Normal .	65.1	34.9	1.1	Minimal traces of choline present.	Nerves irritable and histologically healthy.
1-3 . . .	64.5	35.5	0.9		
4-6 . . .	69.3	30.7	0.9	Choline more abundant.	Irritability lost; degeneration beginning.
8 . . .	68.2	31.8	0.5	Choline abundant.	Degeneration well shown by Marchi reaction.
10 . . .	70.7	29.3	0.3		
13 . . .	71.3	28.7	0.2		
25-27 . .	72.1	27.9	traces	Choline much less.	Marchi reaction still seen, but absorption of degenerated fat has set in.
29 . . .	72.5	27.5	0.0		
44-60. .	72.6	27.4	0.0	Choline almost disappeared.	Absorption of fat complete.
100-106 .	66.2	33.8	0.9	Choline almost disappeared.	Return of function; nerves regenerated.

We see from the foregoing table that the nerves remained excitable up to the third day, and were chemically and histologically healthy. Beyond this date early signs of degeneration set in; the amount of phosphorised material in the nerves slightly dropped, and the amount of choline in the blood slightly

* A full description of this method of phosphorus estimation is given in the *Jour. of Phys.*, 1892, vol. xiii., pp. 814, 821.

increased. On or about the eighth day, the Marchi reaction became strongly marked. This date is coincident with a great drop in the amount of phosphorus in the nerves, and with the appearance of a large quantity of choline in the blood.

The Marchi reaction remained at its acme up to the thirteenth day, and the amount of phosphorus in the nerves became less and less. The amount of choline in the blood was lessened; it therefore appears that of the disintegration products of lecithin, the choline is earliest removed; the phosphorus probably in the form of phosphoric acid next, and the non-phosphorised fat which remains gives the black colour with Marchi's reagent. This fat, however, is absorbed in time.

By the twenty-seventh day, the phosphorus had nearly, and by the twenty-ninth day entirely, disappeared. The removal of the fat had also commenced, so that the particles which stain black with Marchi's fluid were less numerous.

At the forty-fourth day, the removal of the fat was all but complete, and little remained except shrunken empty nerve tubules and connective tissue. This date is, however, variable, for the condition was not so far advanced in another cat sixty days after the nerves had been cut. At any rate, in comparison with the central nervous system, the date of entire removal of fatty particles is an early one.

Regeneration begins about the same time; that is, about the sixtieth day in nerves which had united spontaneously, and a little earlier in cases where the loose ends of the nerves had been sutured together.

By the hundredth to the hundred-and-sixth day regeneration was well marked, especially in sensory fibres, and the nerves were once more excitable. The fibres were seen to be fine, and many were medullated; they took stains normally. Their chemical condition had also almost returned to the normal. The first sign of the return of phosphorus was seen with the commencement of myelination on the sixtieth day, but it was well marked on the hundredth day.

In normal nerves the percentage of phosphorus is a little

over 1 per cent. In the regenerated nerves analysed, it was a little under 1 per cent.

Whether all the phosphorus in the regenerated fibres was in the medullary sheath, or partly in the comparatively large axis-cylinder, it is impossible to say.

With regard to the amount of water in the nerves, the table shows that it increases with the degeneration, and continues high while absorption is occurring. It sinks to the normal when regeneration has set in. The degenerated nerves show to the microscope a loose texture and enlargement of the lymph spaces which will account, in part at any rate, for the increase of water.

Histological Examination of the Nerves

Here I have very little that is new to present to you, though it may interest you to see reproductions of photo-micrographs of our preparations.* The general result of the microscopic study of the nerves will have been gathered from the foregoing summary of our main conclusions.†

I may, however, remind you that in Wallerian degeneration, changes occur in all three parts of a nerve-fibre; the most prominent changes are those seen in the medullary sheath, which undergoes fragmentation into irregular droplets of myelin. It is owing to this circumstance that most of the staining reactions depend upon which we rely to detect degenerated nerve-fibres. The axis-cylinder undergoes a corresponding break-up, and upon this depends the loss of function which occurs. The primitive sheath or neurilemma undergoes a change also. Its nuclei multiply; we noted this in our preparations made from the eight-day cat, but not before that date. In the central nervous system, where the fibres have no primitive sheath, there is naturally no multiplication of its nuclei, but an overgrowth of neuroglia occurs instead. It is possible that the overgrowth of neurilemmal cells in the one case, and of neuroglia in the other, may

* Shown at the lecture as lantern slides.

† Full protocols are given in our original paper in the *Phil. Trans.*

be the result of irritation set up by the products of chemical disintegration.

We have made no special study of the central ends of the divided nerves; this, however, has been done by Noll.* He performed some of his work on large animals (horses), and so could obtain sufficient material from the central end, both for histological purposes and for chemical analysis. Corresponding to what is termed "disuse atrophy," he found some diminution in the amount of phosphorised fat in this region, but the lessening is not so marked as in the peripheral portion of the nerve. He puts the date of disappearance of the phosphorised fat in the peripheral end of the nerve at 28 days; this and many other of his facts fit in very well with our work.

Before passing on to the study of the specimens, I want to detain you first by a description of the Marchi method.

The Marchi reaction consists in placing small pieces of nervous tissue in Marchi's fluid (a mixture of osmic acid and Müller's fluid) after previous hardening in Müller's fluid. Under these circumstances healthy nerve-fibres are stained a greenish-grey colour, but degenerated nerve-fibres are stained an intense black. In the later stages of degeneration, when the fatty products of the decomposition of the fibres have been absorbed, this black staining is naturally no longer observable. It is important, also, to observe that ordinary neutral fats, such as are contained in adipose tissue, give the Marchi reaction. It was knowledge of this fact that led us in part to this investigation, and our expectation has been fully confirmed that accompanying the Marchi reaction in degenerated nerve-fibres is a replacement of the phosphorised fat by non-phosphorised fat.

Before the commencement of our joint work, Dr Mott† had made some preliminary experiments in this direction, which were continued in conjunction with Dr Barratt.‡ Spinal cords, on one side of which degeneration had occurred, due to a lesion in the opposite cerebral hemisphere, were divided longitudinally into two halves; each half was extracted with ether

* *Zeit. f. physiol. Chem.*, vol. xxvii., p. 370.

† Clifford Allbutt's *System of Medicine*, vol. i., *Pathology of Nutrition.*

‡ *Proc. Phys. Soc.; Jour. of Phys.*, vol. xxiv., p. iii.

in a Soxhlet's apparatus. The residue of the ethereal extract from the degenerated side was more abundant, but contained less phosphorus than on the healthy side. The degenerated half of each cord was also more watery.

But the Marchi method may be shortened and simplified by placing the nerve into Marchi fluid direct, without the previous hardening for ten days or so in Müller's fluid.

The result comparing the two methods may be stated in tabular form as follows:—

	Nerve-Fibres.		White Matter of Central Nervous System.	
	Healthy.	Degenerated.	Healthy.	Degenerated.
1. Marchi direct .	Dark greyish-green . . .	Black.	Greyish - green, darker than with nerve-fibres . . .	Black.
2. Marchi, after Müller .	Greyish - green, but not quite so dark . . .	Black.	Greyish - green, like nerve-fibres	Black.

The slight differences of tint noted in the two methods are for practical purposes negligible.

If now you remember what I told you on a previous occasion about osmic acid and fat (p. 67), you will recollect that the reduction of the osmium tetroxide is due to the presence of olein or oleic acid, or some member of the unsaturated series.*

The unsaturated acrylic series of fats represented by olein is capable of reducing osmium tetroxide. Lecithin and other phosphorised fats of the nerve (see p. 67) contain the olein radical, and so reduce the osmium tetroxide. But if the osmic acid is mixed with Müller's fluid, a different result is obtained; the potassium bichromate of the Müller's fluid quickly diffuses into the nerve, and oxidises the olein radical of the lecithin, so that when the more slowly diffusing osmic acid gets in, no call is made upon it to part with its oxygen; hence no reduction of

* See R. Wlassak, *Archiv für Entwickelungsmechanik*, 1898, vol. vi., p. 453; also, Gustav Mann, *Physiological Histology*, p. 315.

the osmic acid takes place, and the nerve does not become black.

In the case of the ordinary neutral fats, such as are found in adipose tissue, the amount of olein is so great, or maybe it is more loosely combined than it is in lecithin, that preliminary or simultaneous treatment with a chromic compound is insufficient to satisfy it, and so osmic acid is reduced, whether it is used alone, or in admixture with Müller's fluid. Both osmic acid and Marchi's fluid therefore produce an intense black staining.

In the case of the degenerated nerve-fibres, the case is more complex. Here the phosphorised fat has been broken up, and the phosphoric acid and choline liberated. Hence the olein which remains is no longer in firm combination, and so is free to behave as it does in adipose tissue. Added to this, we have the further possibility that the olein of the degenerated medullary sheath may be more abundant than it is in healthy lecithin. This, however, has not yet been proved.

We can now pass on to look at some of the photographs of our preparations. Fig. 22 shows the nerve in transverse section two days after it had been cut. It shows the fibrils of the axis-cylinder within the medullary sheath; this nerve was excitable, and normal on microscopic examination. The photograph is necessarily printed in black and white, but it will be remembered that the colour produced by the Marchi stain is a greyish-green.*

The next figure (Fig. 23) of the nerve of a cat a day later, when the nerve had lost its irritability, shows a crinkled outline to the medullary sheaths which, however, still stain greyish-green. The well-defined fibrillæ of the axis-cylinder are no longer distinct.

In the third figure on this plate (Fig. 24) we see a transverse section of the nerve eight days after the operation. There is not a healthy fibre left, and the black staining with the Marchi reagent is quite intense. By this time the percentage of phos-

* Several drawings representing the actual colours of healthy and degenerated fibres treated by the Marchi method will be found in the paper by Mott and myself in the *Phil. Trans.*

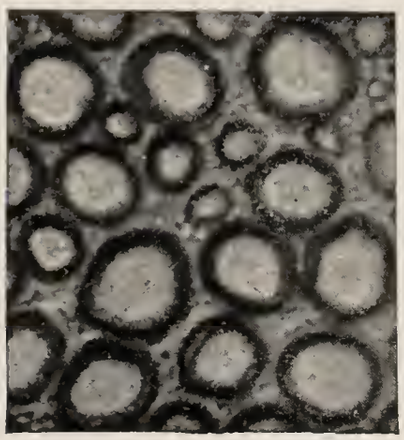

FIG. 22.

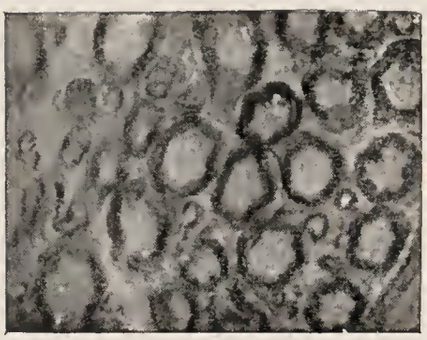

FIG. 23.

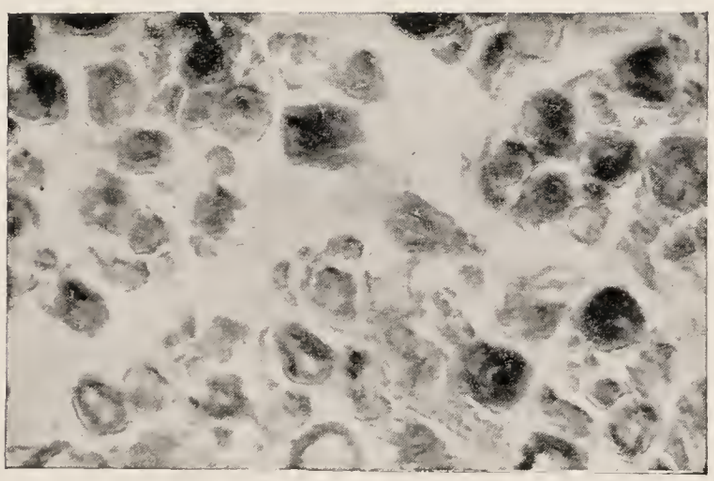

FIG. 24.

FIG. 22.—Transverse section of motor nerve, 53 hours after operation. Method—MARCHI'S fluid direct. In the actual preparation the fibrils of the axis cylinder are well defined. 700 diameters.

FIG. 23.—Transverse section of nerve, 3 days after operation. Same method. 500 diameters.

FIG. 24.—Transverse section of nerve, 8 days after operation. 700 diameters.

[*To face page* 142.

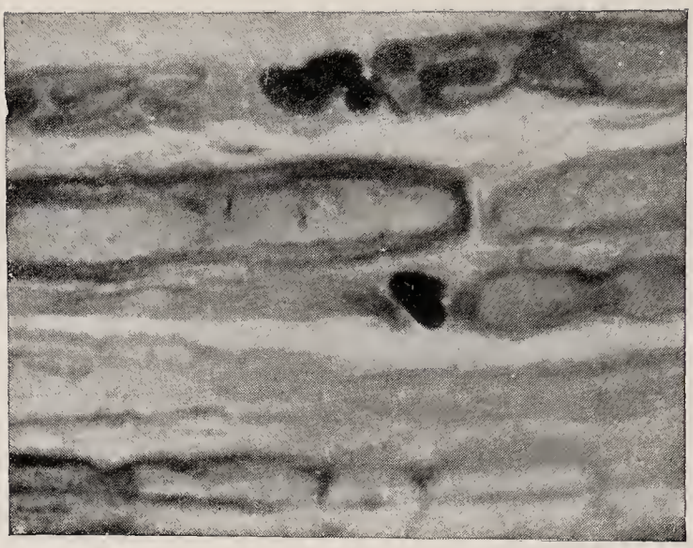

FIG. 25.

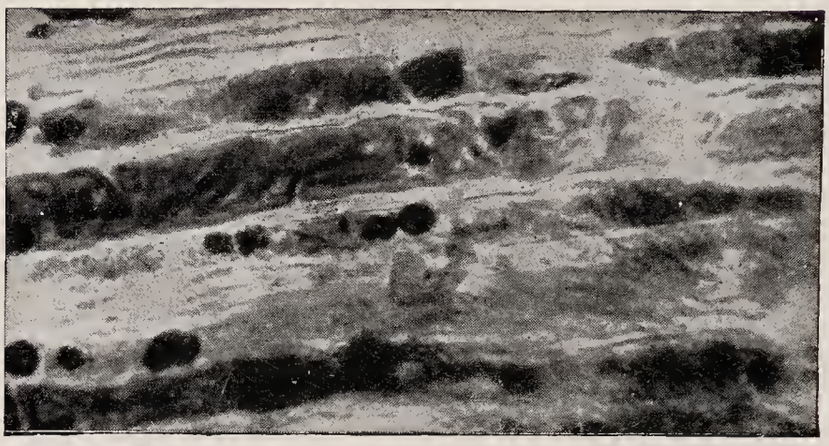

FIG. 26.

FIG. 27.

FIG. 25.—Longitudinal section of nerve, 99 hours after operation. 600 diameters.
FIG. 26.—Longitudinal section of nerve, 10 days after operation. 600 diameters.
FIG. 27.—Longitudinal section of nerve, 27 days after operation.

[*To face page* 143.

phorus had sunk to half the normal. The enlargement of the lymphatic channels alluded to on p. 139 is also well seen.

The three photographs on the next plate show the appearances seen in longitudinal view. Fig. 25 gives an early stage in fragmentation (four days after operation). Fig. 26 shows a much more pronounced condition (10 days after operation); and Fig. 27 a still later stage (27 days after the operation) by which time absorption of the fatty material has begun. The large black spots are fat cells from the neighbouring connective tissue. The exact correspondence in their black colour, with that of the droplets of degenerated nerve fat, is naturally much better seen in the actual specimen, but no doubt can be realised even from the photograph.

The next plate contains four figures, which show the following points:—

Fig. 28 is a single fibre from a teased preparation of a nerve, eight days after it had been cut, stained so as to bring out the division into two of a neurilemmal nucleus.

Fig. 29 is a more highly magnified view of Fig. 27 (27-day cat). It shows a chain of neurilemmal cells filled with fatty particles. These cells, with probably the assistance of phagocytes from the exterior, apparently play an important part in devouring the degenerated fatty material, and so bringing about its removal.

In Figs. 30 and 31 we have a longitudinal and transverse view respectively of a regenerated nerve. The small size of the new fibres will be realised on comparing Fig. 31 with Fig. 22, the magnification in the two cases being the same.

Regeneration of Nerves

Our work on degeneration led us more recently to take up the related question of regeneration.

From the microscopic study of the distal portions of divided nerve-trunks, we arrived at the conclusion that the activity of the neurilemmal cell has some relation to the development of new nerve-fibres. We have seen that at an early stage they multiply (Fig. 28), and later appear to share with phagocytes in

the removal of the broken-up myelin droplets (Fig. 29). Subsequently they elongate, and look as though they were connected end to end, thus leading to the formation of what appear like embryonic nerve-fibres. To suppose that they really form new axis-cylinders would be against the views of Waller and the older physiologists, who taught that the axis-cylinder is essentially the branch of a nerve-cell growing distalwards from the central stump. Among recent writers, Howell and Huber,* who have used both histological and experimental methods of observation, have arrived at the conclusion that although the peripheral structures are active in preparing the scaffolding, the axis-cylinder, the essential portion of a nerve-fibre, has an exclusively central origin. Our own experiments, which have been made on monkeys and cats, are at present incomplete, but the more work we have done on the subject the more have we become convinced that this view is the correct one.

Our preparations appear to us to prove that the manifest activity of the neurilemmal cells is related in some degree, probably nutritionally, to the successful repair of a divided nerve. In situations like the central nervous system, where the neurilemma does not exist, not only is the removal of degenerated myelin a very slow process, but, as is well known, regeneration does not occur.

The elongating and apparently continuous strands of neurilemmal cells to which we have alluded are seen in Fig. 32 in the next plate. It is, doubtless, appearances of this kind which have led some recent observers to the view that new nerve-fibres may have a purely peripheral origin. Ballance and Purves Stewart hold this view. They, however, relied exclusively on histological evidence. One method they employed was Golgi's, which can hardly be considered for this purpose a trustworthy one. It is well known that black streaks are produced by this method by structures which are not nervous at all. A strand that looks like a nerve-fibre is not really such, unless it can be experimentally shown to be excitable and capable of conducting nerve-impulses.

But even careful histological examination will show that the

* *Jour. of Phys.*, 1892-93, vol. xiii., p. 335 ; vol. xiv., p. 1.

FIG. 28.

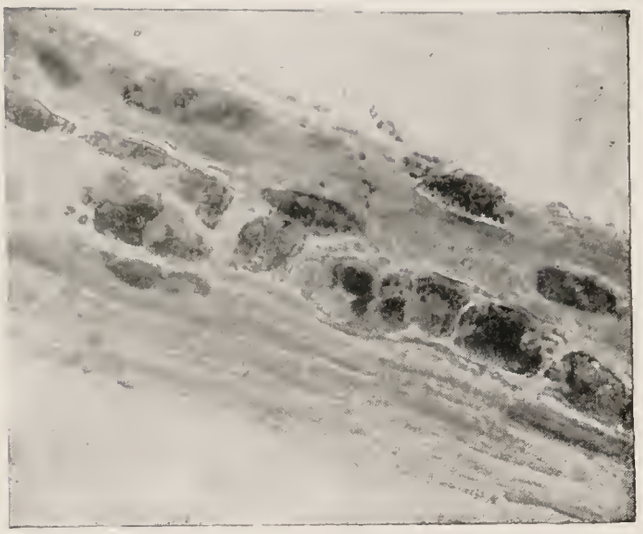

FIG. 29.

FIG. 30.

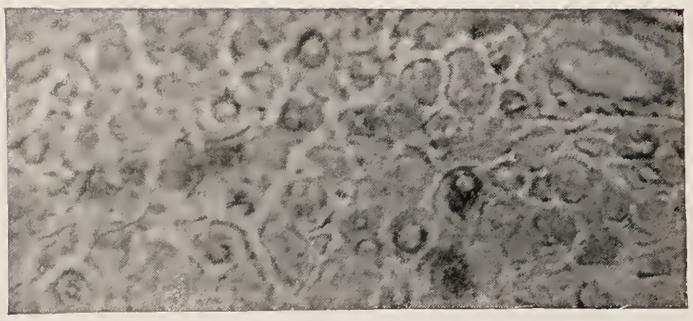

FIG. 31.

FIG. 28.—Single fibre from nerve of cat, 8 days after section. Stained with logwood to show division of nucleus of primitive sheath. 870 diameters.

FIG. 29.—High-power view of figure 27, from 27-day cat. This shows the part played by phagocytes and neurilemmal cells in removing the degenerated fat.

FIG. 30.—Longitudinal section of regenerated sensory nerve, 106 days after operation. 700 diameters.

FIG. 31.—Transverse section of the same. 700 diameters.

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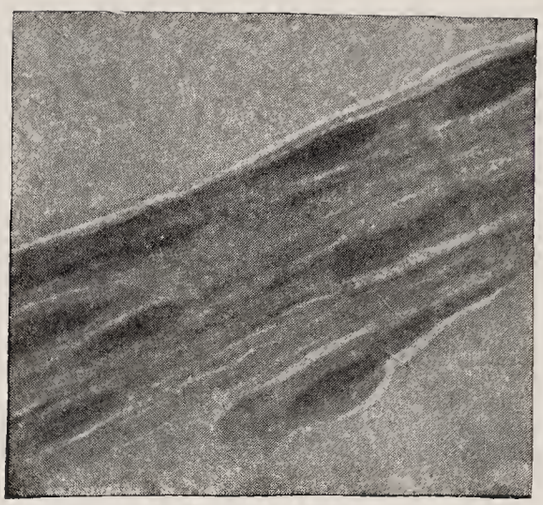

FIG. 32.

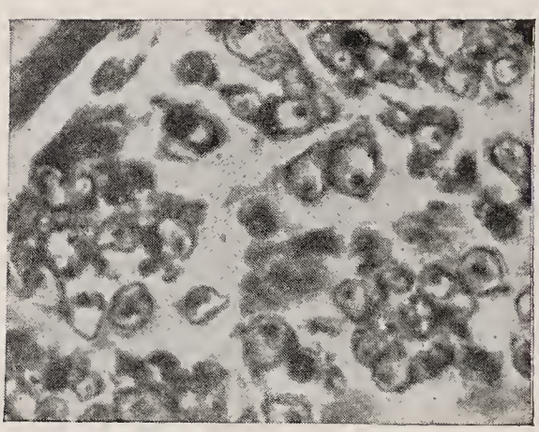

FIG. 33.

FIG. 32.—Longitudinal view of nerve from cat 44 days after the operation; stained with logwood. The elongating neurilemmal cells alluded to in the text are seen. 500 diameters.

FIG. 33.—Transverse section of the regenerated nerve of cat, 100 days after section. Stained by STRŒBE'S method. 700 diameters.

[*To face page* 145.

strands of neurilemmal cells are situated outside the new axis-cylinder, and conceal it underneath or within them; transverse sections of the regenerated nerve (as in Fig. 33) reveal the axis-cylinder quite distinct and separate within the sheath, the nuclei of which retain an abnormal thickness for some time.

The statements of clinical observers, that in man sensation rapidly returns after "freshening up" and suturing together the ends of a nerve which has been divided a long time previously, would be very valuable evidence in favour of the "peripheral theory" if it were entirely trustworthy. A case recently in King's College Hospital was carefully observed, and throws a useful light on this subject. A short time after the operation was performed of uniting the ends of the nerve together, the man stated he was again able to feel, but these sensations rapidly subsided, and sensation did not really return until months later. The preliminary sensation was doubtless subjective; the "freshening up" of the central end of the nerve had evidently caused stimulation of the fibres, which lasted for some hours, and the sensations so produced were referred by the patient's mind to the original terminals of the fibres. Such patients are often hopeful of immediate cure, and this attitude of mind may lead them to assert that recovery has occurred before it has really done so.

Some of the best-attested cases of early recovery of sensation after suture of a previously divided nerve have been recorded by R. Kennedy.* But here, again, the genuineness of the recovery has been doubted; in spite of apparent sensitiveness to such tests as needle-pricks, there is usually, in such cases, absolute anæsthesia to the far more delicate test of stroking the hairs over the affected region.

The great difficulty of obtaining trustworthy evidence from patients has led Dr Head recently to divide and suture certain nerves in his own arm and observe the effects for himself. He has not yet published his results, but from what I have been able to gather, he has noticed no immediate recovery; and the time of return of function has coincided with those of physiological experiments on the lower animals. He also showed the

* *Phil. Trans. Roy. Soc*, 1897, vol. clxxxviii., B., p. 257.

difficulty of localising the stimulus so that it should not affect the hyperæsthetic marginal zone of the anæsthetic region; this probably explains the apparent early recovery to the needle-prick test just alluded to.

Bethe, who is another exponent of the peripheral theory, asserts that the peripheral ends of cut nerves may be excitable without union with the central end; but he does not seem to have excluded a fallacy which was pointed out by Langley and Anderson at a recent meeting of the Physiological Society.* These observers showed that in spite of the absence of any obvious connecting strand with the central end, new nerve-fibres had found their way by devious channels into the peripheral stump from nerves in skin and muscle cut through in the operation.

We have sought to obviate such sources of fallacy by the following means :—

The incisions were made as small as possible, and the parts separated from the nerve-trunks with as little cutting as possible. In cats, one incision over the buttock allowed us to divide the sciatic nerve high up. Another in the ham enabled us to divide the two popliteal nerves. The intervening portion of the sciatic nerve, about 4 or 5 inches long, can be easily pulled out. Additional security to prevent union with central fibres was in some cases obtained by enclosing the upper end of each popliteal nerve in closed caps made out of small drainage tubes about half an inch long. A period of one hundred to one hundred and fifty days was then allowed to elapse, in order that if regeneration was going to occur in the peripheral segments of the nerve, it might have an opportunity of doing so. At the end of this time the animal was anæsthetised, and the nerves tested by electrical stimulation. In all cases they were entirely inexcitable to strong faradic currents, and the wasted muscles also had largely lost their power of response to this form of stimulation. To the naked eye the nerves were pale. The animals were killed, and microscopical investigation of the nerves showed no trace of regeneration. In those cases where

* *Proc. Phys. Soc.*, 13th December 1902. *Jour. of Phys.*, vol. xxix., p. ii. See more fully, *ibid.*, 1904, vol. xxxi., p. 418.

the nerves had been placed in tubes, it was very difficult to recognise any nervous structure whatever.

Another experiment suggested to us by Professor Gotch has been performed both on the monkey and cat. A large nerve was divided, and the ends sutured together. After a sufficient length of time had elapsed, restoration of function led us to suppose that regeneration had occurred. The nerve was exposed; the union of the two ends was found to have been accomplished, and the nerve was excitable both above and below the junction. A piece of nerve was then excised an inch or so below the junction, and on histological examination of this, all traces of degenerated products were found to have disappeared, and it was made up of fine new nerve-fibres, many of which had acquired a delicate medullary sheath. After this second operation, the wound was closed and the animal allowed to live for ten days longer. It was then killed, and the nerve both below and above the second cut was then examined. No degeneration was found in the nerve-fibres above the second lesion, but Wallerian degeneration was shown by the Marchi method to have occurred in medullated fibres of the peripheral portion, which was quite inexcitable. The direction of degeneration is the direction of growth; so this experiment shows that the growth of the new fibres had not started from the periphery centralwards, but in the reverse direction.

Another piece of evidence bearing in the same direction consisted in examining regenerated nerve-fibres in various parts of their course. We think in some cases that the more distant the situation from the original point of section, the less perfectly developed the new fibres appear to be; myelination has progressed less in the distal portion of their course. But further observations on this point are being made.

Another set of experiments consisted in attempting to ascertain the influence of stimulus on regeneration. A monkey's arm was rendered immobile by the division of a number of the upper posterior roots. The anterior cornual cells, from which the corresponding motor fibres originate, are thus not subjected to stimuli from the periphery; and, as Mott and Sherrington were the first to show, the arm is as much paralysed as if the

anterior roots had been cut. We have, however, again noticed in some of these animals under the influence of strong emotion (for instance, when the monkey is prevented from reaching with the sound hand a piece of apple), that some efforts are made to move the other limb. When the animal is living in its cage under ordinary conditions, it makes no effort to move the limb, which in successful experiments (*i.e.* when a sufficient number of roots have been entirely cut through) hangs helpless like a flail. A large nerve in the arm (median or ulnar) was then divided, and the ends sutured together; the corresponding nerve was divided and sutured on the non-paralysed side as a control experiment. The animal was finally killed; the interval between the operation and death varied in different experiments, but the best time for making the observation we finally determined to be between sixty and seventy days after the nerves had been cut.

Union of the divided nerves occurs on both sides of the body, and in our early experiments, the nerve on the side corresponding to that on which the posterior nerve-roots had been divided was found to be less excitable to the faradic current; histologically, this nerve showed a looser texture, and new nerve-fibres, though present, were somewhat less numerous than on the control side. In these early experiments, also, we found that the posterior cornual cells in the cervical region were atrophied, and that there was a considerable overgrowth of neuroglia tissue in the posterior horn.

Further examination (by the methylene-blue and erythrosin stain) of these spinal cords showed, however, that there had been a considerable number of small hæmorrhages, sufficient in some cases to cause degeneration in various descending tracts in the cord. It therefore became quite possible to explain the effects observed by this complication. We are inclined to think that the hæmorrhages are not due to mechanical injury of the cord during the operation, but are to be explained by the loss of support in the cord tissue which follows degeneration of the entering posterior root-fibres.

In several of the later experiments, in which the cord

hæmorrhages did not occur to any great extent, we have been unable to detect any marked changes in the posterior cornual cells, or any difference to stimulation or in microscopical structure between the regenerated nerves of the two sides.

This result accords with some experiments carried out by Anderson * on developing animals; he found that section of all the posterior roots connected with a limb caused no retardation in the development of the corresponding anterior roots.

In further experiments we sought to cut off the cerebral influence by removing the cortical arm area of the opposite side, in addition to dividing posterior roots as before. In this case, also, the regenerated nerves of the two sides were equally responsive to stimulation, and histological evidence of any marked difference between them was also lacking.

At present we are engaged in performing experiments in which a transverse section of the cord is combined with division of posterior nerve-roots. By this means we hope to still further reduce the action of innervation currents on the anterior cornual neurons by cutting off the stimuli which enter by the posterior roots, as well as those which descend from the brain. It is quite possible that the paths which will even under those circumstances remain open (commissural and association tracts) may be sufficient to maintain the activity of the anterior cornual cells in the sprouting forth of new axons in a peripheral nerve, though they may be insufficient to induce those cells to send effective impulses along them.

Warrington has stated that when posterior nerve-roots are cut, the anterior nerve-cells undergo the chromatolytic change associated with inactivity. Warrington's observations, however, were made at an early date after the division of the roots. In the animals which we have killed at the late dates mentioned, it was not possible with any certainty to tell by looking at the anterior horn cells of the two sides which was the side on which the posterior roots had been divided.

Another question, and one which is of equal interest in any I have mentioned, is the mode of origin of the medullary sheath.

* *Jour. of Phys.*, vol. xxviii., p. 499.

This, however, I must only touch upon. The activity of the neurilemmal cells is the only point in favour of the view that it is formed from them. All the other facts point to the origin of the medullary sheath from the axis-cylinder, and this evidence, which to me seems conclusive, is :—

1. In a developing or regenerating nerve-fibre, complete functional activity is associated with the appearance of the medullary sheath.

2. When a nerve is cut, the medullary sheath is a part which markedly shares in the degenerative process.

3. The medullary sheath appears in the nerve-fibres of the central nervous system, that is, in a portion of their course where the primitive sheath is absent.

The axis-cylinder and its sheaths must necessarily, for descriptive purposes, be considered separately. There is little doubt in my own mind, that, functionally, all three parts of a nerve-fibre must be considered to act as an organic whole, with intimate inter-relations of a nutritional or metabolic nature.

The statements by Bethe, Dohrn and others concerning the peripheral origin of new nerve-fibres has called forth a vigorous protest from the veteran histologist, Kölliker, in a recent number of the *Anat. Anzeiger* (vol. xxv., p. 1). He points out that Ramon y Cajal's observations support the view of the central origin of nerve-fibres, which he, among others, enunciated many years ago. The process of the nerve-cell which we call the axon becomes differentiated into axis-cylinder and medullary sheath. The investing cells that form the neurilemma are mesoblastic in origin.

It would on *à priori* grounds be extremely improbable that mesoblastic cells should be capable of forming new axis-cylinders, and assuming the highly-specialised function of conducting nerve impulses in place of the original axis-cylinders, which have an epiblastic origin.

This brings me to the end of what I have to say. The researches are many of them unfinished, but, nevertheless, my lectures must terminate; and I have in conclusion to express my gratitude to those who have given me the opportunity of stringing my facts together in this way, and to you who have patiently and attentively followed my utterances.

In the conventional works of fiction, the author is able to manipulate his puppets in such a way that the wind-up is satisfactory, and all live happily ever afterwards. In actual life,

things seldom happen so as to terminate in a round-up of this kind. So it is with science; its great charm is that it never finishes. We call a halt now and then and report progress, but work goes on, new discoveries are made, and every real discovery opens the road to others, and still others beyond those.

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PRINTED BY OLIVER AND BOYD, EDINBURGH.